Recommended Protocols for Measuring Conventional Water Quality Variables and Metals in Fresh Water of the Puget Sound Region

For

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ACKNOWLEDGMENTS

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INTRODUCTION

This document presents guidance on the sampling and analysis of freshwater environments in the Puget Sound region. The freshwater environments considered include lakes, rivers, and streams. Sampling and analysis of municipal wastes, industrial effluents, and brackish waters are not included in this document. Methods for sampling selected conventional variables [e.g., flow, dissolved oxygen (DO) concentration] and metals (e.g., silver) are summarized.

The recommended methods are based on the results of written reviews by representatives from organizations that fund or conduct environmental studies in the Puget Sound region, and a workshop sponsored by the Puget Sound Estuary Program (PSEP) which was held on 18 August 1988. Workshop participants are listed in Table 1.

The purpose for developing these guidelines is to encourage all investigators in the Puget Sound region conducting monitoring programs, baseline surveys, and intensive investigations to use standardized methods and reporting techniques whenever possible. If this goal is achieved, then most data collected in the region should be directly comparable, and thereby capable of being integrated into a sound-wide database. Such a database is necessary for developing and maintaining a comprehensive water quality management program for the Puget Sound region.

The sections on conventional variables and metals are presented separately. Each recommended method describes the use and limitations of the respective variable; the field collection and processing methods; and the analytical, quality assurance/quality control (QA/QC), and data reporting procedures for the laboratories. Specific data quality objectives are not included in the protocols, because generally they are specific to various programs.

In developing the recommended protocols, it was recognized that the methods used in the study of the freshwater environments continuously change. The loose-leaf format of this document will allow modification of the recommended methods in the future, and if necessary, the inclusion of additional methods.

Although the following methods are recommended for most studies conducted in Puget Sound, departures from these methods may be necessary to meet the special requirements of individual projects. However, if such departures are made, then the funding agency or investigator should be aware that the resulting data may not be comparable with similar data. In some instances, data collected using different methods may be compared if the methods have been adequately intercalibrated.

TABLE 1. ATTENDEES OF FRESHWATER PROTOCOLS WORKSHOP

Name	Affiliation
John Bernhardt	Washington Department of Ecology (Ecology), Technical Services
Perry Brake	Ecology, Quality Assurance Section
Steve Brown[a]	Tetra Tech, Inc.
Isabel Chamberlain	EPA Manchester Laboratory
Eric Crecelius	Battelle Pacific Northwest Laboratories
John Dailey	Am Test, Inc.
Mark Fugiel	Am Test, Inc.
Rich Horner	University of Washington
Dick Huntamer	Ecology, Manchester Laboratory
Jean Jacoby	Tetra Tech, Inc.
Cheryl Kamera	Municipality of Metropolitan Seattle (Metro)
Wayne Kraft	Ecology, Manchester Laboratory
Lawrence McCrone	Ecology/EPA Office of Puget Sound
Joy Michaud	Ecology, Surface Water Investigations
Ann Peacock	Metro
Earl Skinner	U.S. Geological Survey
Dimitri Spyridakis	University of Washington
Bruce Woods	EPA Manchester Laboratory

[a] Moderator.

Recommended methods for measuring the following conventional water quality variables in fresh waters in the Puget Sound region are presented in this document:

- Flow
- Temperature
- Conductivity
- Dissolved oxygen
- pH
- Alkalinity
- Total hardness
- Total suspended solids
- Turbidity
- Ammonia-nitrogen
- Nitrate+nitrite-nitrogen
- Total phosphorus
- Orthophosphate-phosphorus
- Fecal coliform bacteria.

Metals are discussed later (see *Recommended Methods for Measuring Metals*).

Each method is based on a review of the practices of federal, state, and local agencies, and university and private laboratories active in monitoring the fresh waters of the Puget Sound region. The high degree of consensus in the methods used among these organizations is reflected in the methods presented here. An exception to this consensus is the degree of laboratory automation for nutrient analyses. Some organizations have converted from manual to automated methods, while others are in the process of conversion.

Many of the methods used in the Puget Sound region are contained in several general documents listed in Table 2 that are commonly used among these organizations. The procedures described herein were adopted from one or more of these sources. The original documents remain valuable references to provide detail.

TABLE 2. GUIDANCE DOCUMENTS USED BY ORGANIZATIONS PERFORMING WATER QUALITY MONITORING IN FRESH WATER IN THE PUGET SOUND REGION

APHA. 1989. Standard methods for the examination of water and wastewater. 17th Ed. American Public Health Association, Washington, DC.

Huntamer, D. 1986. Department of Ecology laboratory user's manual. Washington Department of Ecology, Olympia, WA.

METRO. Several dates. Laboratory methods. Municipality of Metropolitan Seattle, Seattle, WA.

U.S. EPA. 1978. Microbiological methods for monitoring the environment, water, and wastes. EPA-600/8-78-017. U.S. Environmental Protection Agency, Cincinnati, OH.

U.S. EPA. 1979a. Handbook for analytical quality control in water and wastewater laboratories. EPA-600/4-79-019. U.S. Environmental Protection Agency, Cincinnati, OH.

U.S. EPA. 1979b. Test methods for evaluating solid waste, physical/chemical methods, SW-846. Third Edition. U.S. Environmental Protection Agency, Office of Solid Waste, Washington, DC.

U.S. EPA. 1982. Handbook for sampling and sample preservation of water and wastewater. EPA-600-82-029. U.S. Environmental Protection Agency, National Environmental Research Center, Cincinnati, OH.

U.S. EPA. 1983. Methods for chemical analysis of water and wastes. EPA-600/4-79-020. U.S. Environmental Protection Agency, Cincinnati, OH.

U.S. EPA. 1987a. Contract Laboratory Program. Statement of work for organic analyses. U.S. Environmental Protection Agency, Sample Management Office, Alexandria, VA.

U.S. EPA. 1987b. Contract Laboratory Program. Statement of work for inorganic analyses. U.S. Environmental Protection Agency, Sample Management Office, Alexandria, VA.

USGS. 1977. National handbook of recommended methods for water-data acquisition. U.S. Department of the Interior, U.S. Geological Survey, Office of Water Data Coordination, Reston, VA.

USGS. 1985. Methods for the determination of inorganic substances in water and fluvial sediments. Open File Report 85-495. Fishman, M.J., and L.C. Friedman (eds). U.S. Geological Survey, Denver, CO.

GENERAL CONSIDERATIONS FOR SAMPLING AND ANALYZING CONVENTIONAL VARIABLES

The essential tasks in sampling freshwater environments are to obtain a sample that meets the requirements of the program and to prevent deterioration and contamination of the sample before and during analysis. Several general recommendations are presented in this section to aid in accomplishing these tasks. Specific guidelines are provided in the sections for the individual variables. These recommendations do not address all possible sampling situations.

TYPES OF SAMPLING

Grab samples are collected at a discrete point in time and space. Composite samples are made by combining a number of samples taken at different locations and/or different times. For flowing water sampling, flow-proportional composited samples are more representative of average water conditions than grab samples or samples taken at certain time intervals that are composited without regard to flow. Integrated samples refer to spatial composites. While composites can be taken over any dimension, compositing over depth is most common. In variable-depth composites, a series of grab samples should be combined in proportion to flow velocities over the depth profile.

Sampling can be performed manually or with automatic collectors. Automatic collectors are available that can collect a series of discrete samples, time-proportional, or flow-proportional composites. Flow measurements, which are required to produce flow-proportional composites, can be performed manually or with a continuously recording meter.

Manual grab sampling with flow estimation is the most common approach used in monitoring ambient water and will be emphasized in this document. Manual compositing is generally inconvenient, but can be performed occasionally for special purposes. Automatic samplers and flow recorders are relatively expensive, need considerable maintenance, are vulnerable to damage in the field, and require experienced personnel for operation. Use of these devices requires a commitment by the investigator to address these issues.

RECOMMENDATIONS FOR MANUAL GRAB SAMPLING

The principal problem in manual grab sampling is to obtain a sample that is representative of the conditions being investigated.

Sampling Locations

The guidelines for obtaining representative samples differ somewhat for flowing and standing water.

Streams and Rivers—In the case of streams and rivers, it usually must be assumed that relatively homogeneous conditions prevail over the width and depth dimensions of the water. Small systems are generally more homogeneous than large systems. As a result of this assumption, samples for water quality variables in streams and rivers are usually collected at midstream and at one depth. In the absence of any special considerations, collection at half of full depth is recommended. (Refer to specific guidelines for the particular variables of interest for exceptions to this recommendation.) Environmental conditions in streams and rivers differ longitudinally and with changing flows. Therefore, sampling programs often require multiple stations and sample collection in a range of flow conditions in dry and wet weather. Additional guidance on selection of sampling stations and sampling frequencies in Washington is available in Horner et al. (1986).

Lakes and Reservoirs—Lakes and reservoirs are often assumed to be relatively homogeneous horizontally, unless they are large or heavily indented with bays. However, thermal stratification in all but the most shallow systems can cause substantial variation in environmental conditions with depth. Moreover, thermal stratification varies seasonally. In the Puget Sound region, lakes are monomictic (i.e., they stratify thermally from late spring to late fall and mix from top to bottom through the winter and most of the spring), except at high elevations. Lakes at high elevations are dimictic (i.e., they are thermally stratified during two periods per year, winter and summer). During the warm season, stratified lakes typically have three distinct layers: a relatively uniform warm upper layer (epilimnion), a cold lower (hypolimnion) layer, and a transition layer between (metalimnion). The metalimnion exhibits a temperature gradient. At high elevations during the cold season, the epilimnion is the coldest layer.

Sampling depths must be selected based on program objectives. A typical program in a small, stratified lake would encompass development of temperature, DO, and specific conductivity profiles over depth, and grab sampling for other variables at one mid-lake station at 0.5 to 1-m depth (to avoid surface scums). A second depth in the epilimnion may be selected if this layer is relatively thick. If there is interest in release of chemicals (e.g., nutrients, metals) from the sediments, a hypolimnetic sample should also be taken.

Representative sampling of a large lake may require collection of several samples along a transect or in different basins. Sampling during the growing season (i.e., April to October in the Puget Sound region) is often emphasized because of the biological activity and potential hypolimnetic oxygen depletion that occur during this period. Fewer samples are taken in the winter. Further information concerning lake sampling is available in Cooke et al. (1986) and Vollenweider (1974).

Sample Collection

Samples can be collected from a bridge or a boat using one of several designs of specialized samplers or by wading and dipping a sample bottle beneath the surface at the selected point of sampling. The specialized samplers are typically cylinders lowered into the water with both end closures held open. When the sampler reaches the desired depth (determined from a marked line attached to the sampler), a messenger is dropped down the line to trip the closure mechanism. The sampler is drained through a spigot into sample bottles for onsite analyses and transport to the laboratory. A commonly used sampler of this type is the Van Dorn bottle. Samples may be obtained in sites with rapid currents by lowering a weighted, stainless steel bucket to a depth of 30 cm below the surface.

When wading, the individual collecting samples should face upstream. This orientation minimizes contamination of the sampled water that would be caused by the sampler's presence. The container should enter the water with the opening down to minimize collection of material from the surface layer.

Unless a preservative has been added to the sample bottle before collection, it should be rinsed with two or three volumes of water before capping. Rinsing can be accomplished by overflowing from a Van Dorn or similar sampler. When the investigator is wading, bottle rinsing can be accomplished by filling and totally emptying the sample bottle several times.

In a continuing program, the same containers could be reused for the same stations. This practice minimizes potential contamination of relatively clean samples when highly polluted samples might have been collected previously in the same containers. Nevertheless, sample containers should always be cleaned thoroughly as recommended below in the *Cleaning Methods* section. A single container can typically be used to hold samples that will be analyzed for several variables with compatible preservatives. For example, conductivity, pH, alkalinity, total hardness, total suspended solids, and turbidity analyses can usually be performed on samples from one container, and nutrient analyses can usually be performed on samples from a second container (Table 3).

When visiting a sampling station, the sample collector should record the following information in a field book:

- Date
- Time
- Name of individual collecting sample
- Number of samples collected
- Weather and flow conditions
- Onsite field measurements (e.g., temperature)

TABLE 3. RECOMMENDED SAMPLE SIZES, CONTAINERS, PRESERVATION TECHNIQUES, AND HOLDING TIMES FOR MEASUREMENT OF CONVENTIONAL WATER QUALITY VARIABLES

Variable	Minimum Sample Size (mL)[a]	Container[b]	Preservation	Holding Time[c] Recommended	Maximum
Temperature	1,000[d]	P,G	None	Zero[e]	Zero[e]
Conductivity	100	P,G	Cool, 4° C[f]	28 days	28 days
Dissolved oxygen	300	G (BOD bottle)	Fix with reagents, store in dark	8 hours	8 hours
pH	25[g]	P,G	None	Zero[e]	Zero[e]
Alkalinity	100	P,G	Cool, 4° C[f]	24 hours[h]	14 days
Total hardness	100	P,G	HNO_3 to pH<2	6 months	6 months
Total suspended solids	1,000[i]	P,G	Cool, 4° C[f]	7 days	7 days
Turbidity	100	P,G	Cool, 4° C[f]	24 hours	48 hours
Ammonia-nitrogen	125	P,G	H_2SO_4 to pH<2 Cool, 4°C[f]	7 days	28 days
Nitrate + nitrogen-nitrogen	125	P,G	H_2SO_4 to pH<2 Cool, 4°C[f,j]	24 hours	28 days
Total phosphorus	50	P,G	H_2SO_4 to pH<2 Cool, 4°C[f]	48 hours	28 days
Orthophosphate-phosphorus	50	P,G	Filter on site Cool, 4° C[f]	24 hours	48 hours
Fecal coliform bacteria	125	P,G[k]	Cool, 1-4° C[f]	6 hours	30 hours

[a] Recommended field sample size for one laboratory analysis of the given variable.

[b] P - Polyethylene, polypropylene, or fluoropolymer; G - glass.

[c] Analyze within the recommended time if possible, but in all cases within the maximum. The holding times given are for routine monitoring work. Research objectives may require shorter holding times.

TABLE 3 continued

[d] Measuring directly in water body is preferred.

[e] Analyze immediately.

[f] Holding at 4° C implies holding in the dark.

[g] Increase the volume to rinse the pH electrodes several times, especially in low-alkalinity waters.

[h] It is preferred by some agencies to analyze low-alkalinity waters in the field, while greater analytical control available in the laboratory is preferred by other agencies.

[i] Volume given is the maximum needed to filter for analysis of low concentrations. A smaller quantity (100-250 mL) is adequate for most samples.

[j] If nitrate-nitrogen data are needed, a separate, nonacidified sample is required. The nonacidified sample must be analyzed for nitrite-nitrogen within 48 hours. Nitrate-nitrogen is determined by subtracting nitrite-nitrogen from nitrate+nitrite-nitrogen.

[k] Container must be able to withstand autoclaving at 121° C for 20 minutes.

- Unusual conditions (e.g., oil on the water; water coloration or turbidity; fish kill; changes in nearby land use, aquatic and riparian vegetation)

- Calibration results for field instruments.

Sample Heading

To avoid mistakes, it is imperative to label a sample bottle with an indelible marker at or before the time of collection. It is most efficient to prepare and attach labels before going into the field. Sample labels must include station designation, date, time, collector's name, and any preservative added. The analyses to be performed and any pertinent remarks may also be recorded on the label.

It is recommended that a sample tracking record be kept for each sample. This record registers possession of a sample as it travels from collection through analysis, which may allow misplaced samples to be found more readily. A typical sample tracking record form is illustrated in Figure 1. Samples that may be involved in litigation may require formal sample tracking records, termed chain-of-custody records.

Samples must be preserved and analyzed within a certain period to avoid deterioration. Recommended preservation methods and holding times are given in Table 3. These recommendations were derived from American Public Health Association (APHA) (1985), U.S. EPA (1983), and discussions at the workshop.

Sample Size

A minimum sample size of at least 2.5 times the recommended sample volumes given in Table 3 should be collected whenever possible. The volumes given in Table 3 are sample volumes required for a single analysis of each variable. Additional sample may be needed for rinsing instrument sensors and for possible repeated analyses. Repeated analyses may be needed for analyzing replicates and for reanalyzing samples when (QA/QC) criteria are not met (see below).

ANALYTICAL METHODS AND DETECTION LIMITS

Recommended analytical methods and detection limits for conventional variables are given in Table 4. Detection limits typically achieved using the recommended methods are also given in Table 4.

QUALITY ASSURANCE/QUALITY CONTROL GUIDELINES

The effectiveness of any monitoring effort depends on its QA/QC program. The QA/QC program provides quantitative measurements of the "goodness" of the data. For some variables, QA/QC may involve calibration

SAMPLE TRACKING FORM

				SAMPLERS ***(Signature)***					
STATION NUMBER	STATION LOCATION	DAY	TIME	SAMPLE TYPE Water *Comp.*	 *Grab*	 Air	SEQ. NO.	NUMBER OF CONTAINERS	ANALYSIS REQUIRED

Relinquished by: *(Signature)*	Received by: *(Signature)*	Date/Time
Relinquished by: *(Signature)*	Relinquished by: *(Signature)*	Date/Time
Relinquished by: *(Signature)*	Received by: *(Signature)*	Date/Time
Received by: *(Signature)*	Received by Mobil Laboratory for Field Analysis: *(Signature)*	Date/Time
Dispatched by: *(Signature)* Date/Time	Received for Laboratory by: *(Signature)*	Date/Time
Method of Shipment:		
Distribution Orig: Accompany Shipment 1 Copy – Survey Coordinator Field Files		

Figure 1. Typical sample tracking record form

TABLE 4. RECOMMENDED ANALYTICAL METHODS FOR CONVENTIONAL VARIABLES

Variable	Unit	Recommended Detection Limit	Recommended Analytical Methods
Flow	m^3/sec	--	Current meter survey Staff gauge
Temperature	° C	--	Mercury-filled thermometer Digital probe
Conductivity	µmhos/cm[a]	1	Conductivity meter
Dissolved oxygen	mg/L	--	Azide-modified Winkler Membrane electrode
pH	pH units	--	Electrometric
Alkalinity	mg/L as $CaCO_3$	1	Titrimetric
Total hardness	mg/L as $CaCO_3$	1	EDTA titrimetric
Total suspended solids	mg/L	--	Gravimetric
Turbidity	NTU[b]	1	Nephelometric
Ammonia-nitrogen	µg/L	10	Automated phenate[c] Phenate[c]
Nitrate + nitrite-nitrogen	µg/L	10	Automated cadmium reduction Cadmium reduction[c]
Total phosphorus	µg/L	5	Automated ascorbic acid reduction[c] Heteropoly blue ascorbic acid (following persulfate digestion)[c]
Orthophosphate-phosphorus	µg/L	2	Automated ascorbic acid reduction[c] Heteropoly blue ascorbic acid[c]
Fecal coliform bacteria	colonies/ 100 mL	1	Membrane filter

[a] Millisiemens/meter (mS/m) are used in the SI system. 1 mS/m = 10 µmhos/cm.

[b] NTU - Nephelometric turbidity units.

[c] Both automated and nonautomated procedures are recommended for nutrient analyses because some laboratories have not been converted to automated techniques.

of instruments with known standards. To obtain measures of accuracy and precision, QA/QC may further involve analysis of blanks, replicate samples, control samples, and spiked samples. Definitions of terms that apply to the measurement of the conventional water quality variables covered in this document are given below.

QA/QC guidelines for sampling and analysis of conventional variables are given in Table 5. The sections on each variable contain additional information, including accuracy and precision data. Specific QA/QC requirements should be stated explicitly in any contract. Discussions among project managers and field and laboratory personnel concerning the QA/QC requirements of a project should be conducted before a contract is signed. Requirements may differ among projects. For example, a project involving enforcement actions or litigation may have more stringent QA/QC requirements than a project involving routine ambient monitoring. More information is available in U.S. EPA (1979a) and APHA (1985).

Definitions

Field Replicates—Field replicates are separate samples collected simultaneously at the identical source location and analyzed separately. Field replicates are used to assess total sample variability (i.e., field plus analytical variability).

Laboratory Replicates—Laboratory replicates are repeated analyses of a variable performed on the contents of a single sample bottle. Laboratory replicates are used to assess analytical precision. Usually duplicate analyses are sufficient for procedures that are well proven in the laboratory.

Calibration Standards—A sample prepared from distilled-deionized water that contains a known concentration of a specific substance or will produce a known instrument response is a calibration standard. The distilled-deionized water used in calibration samples should meet Type 1 water quality criteria specified by APHA (1985) Method 107.4.

Blanks—A blank is a sample prepared from Type 1 water (resistivity > 10 megohm-cm), perhaps with reagents added, to represent zero concentration of a specific substance, or to produce an instrument response that indicates zero concentration.

A transport blank may be useful in studies of nutrients, fecal coliform bacteria, and metals. A transport blank is a blank that is transported to the sampling location and treated like a sample thereafter.

Spiked Samples—A sample prepared by adding a known concentration of a specific substance to an environmental sample is called a spiked sample.

TABLE 5. CALIBRATION AND QUALITY ASSURANCE/QUALITY CONTROL GUIDELINES FOR MEASUREMENT OF CONVENTIONAL WATER QUALITY VARIABLES

Variable	Guidelines[a]
Temperature	Check thermometer against a thermometer certified by American Society for Testing and Materials or National Bureau of Standards.
Conductivity[b]	Calibrate in the laboratory with two standard KC1 solutions repre-senting the expected conductivity range of the samples. Check calibration using one standard KC1 solution (with conductivity in the sample range) per batch in the laboratory or whenever the meter is set up in the field.
Dissolved oxygen[b]	For the azide-modified Winkler method, run one 100 percent satur-ated calibration sample/batch. For studies where low DO concentra-tions are expected, a calibration sample containing zero DO may be used. For the membrane-electrode method, calibrate with a sample of known DO concentration (determined using the azide-modified Winkler method) and with a sample containing zero DO. Calibration is required prior to the start of every series of measurements and whenever the meter is moved or turned off.
pH[b]	Calibrate with two buffers. Check calibration with a third buffer every 3 hours. Use neutral, acid, and basic buffers (e.g., pH 4.0, 7.0, and 10.0) prepared according to National Bureau of Standards Special Publication 260-53 (Durst 1975).
Alkalinity[b]	Calibrate pH meter as above. Check titrant normality with self-prepared and EPA standard solutions (1 check/batch).
Total hardness[b]	Check titrant molarity with self-prepared and EPA standard solutions (1 check/batch) and run one blank/batch. Run one spiked sample/batch if interference is suspected.
Total suspended solids[b]	Check balance calibration monthly and oven temperature daily. Balances should have annual preventative maintenance checks. Run at least one EPA or commercial control suspension of known concentration per set of 20 samples.
Turbidity[b]	Calibrate with commercial standard in same range as samples. Recalibrate with every range change.
Manual nutrients[b]	Run calibration curve with a blank and standards at 0.2, 0.35, 0.5, 0.75, and 1.0 cu[c]. Entire range of sample concentrations must be included in the calibration curve. Run control samples at 0.2 and 0.9 cu with each batch. Run two blanks/batch and one spiked sample/batch.
Automated nutrients[b]	Run calibration curve with a blank and standards at 0.2, 0.5, and 1.0 cu. Entire range of sample concentrations must be included in the calibration curve. Run control samples at 0.2 and 0.9 cu with each batch. Run two blanks/batch and one spike sample/batch.

TABLE 5. (Continued)

Variable	Guidelines[a]
Fecal coliform bacteria	Laboratory replicates should be analyzed at a frequency of 10 percent.

[a] A batch is defined as a group of no more than 20 samples.

[b] Field replicate samples should be collected and analyzed at a frequency of 5-10 percent. Laboratory replicates should be analyzed at a frequency of 5-10 percent.

[c] cu - upper limit of expected concentration range.

Accuracy—Accuracy is the measure of agreement between the measurement of a variable in a sample and the true value of the variable in that sample. The term "error" is used when the discrepancy between the measured and true values is expressed in the units of the measured variable. The term "relative error" is used when the error is expressed in terms of the percentage deviation from the true value.

Precision—The measure of agreement among replicate laboratory measurements is called precision. Precision is measured by the standard deviation when the units of the measured variable are used. The term "relative standard deviation" is used when the standard deviation is expressed as a percentage of the mean of the replicate values.

Criteria for Acceptance of QA/QC Results and Corrective Actions

This section contains general guidelines for acceptance of QA/QC results and corrective actions. More detailed information concerning QA/QC results and corrective actions is available in the references cited in the following sections on each variable and in U.S. EPA (1979a).

Control limits for accuracy and precision are established in every laboratory, and these limits may vary among laboratories. Accuracy and precision data are presented in the following sections for each variable. This information was obtained from the cited references and is presented only to provide general guidance for accuracy and precision. More detailed information concerning the accuracy and precision data is available in the cited references.

Check Standards—If the results of the analysis of a control sample fall beyond the control limits that are established by the laboratory or specified in the contract, the analysis should be terminated, the problem causing the analysis to be beyond the control limits identified and corrected, and the analyses repeated. The control limits suggested by U.S. EPA (1979a) and APHA (1985) are ±3 times the standard deviation for analysis of a control sample. This standard deviation should be calculated from at least 20 separate analyses of control samples.

Blanks—Rerun analysis of the affected samples if the blank measurement exceeds the method detection limit (see Table 4).

Spiked Samples—The results of the analysis of a spiked sample should be compared with control charts established by the laboratory for spiked sample analysis. Control charts are discussed in U.S. EPA (1979a). If the results are beyond the established control limits, the analysis should be terminated, the problem identified and corrected, and the analyses affected by the problem repeated.

Replicates—The results of the analysis of laboratory replicate samples should be compared with control charts established by the laboratory for replicate sample analysis. Control charts are discussed in U.S. EPA (1979a). If the results are beyond the established control limits, the analysis should be terminated, the problem identified and corrected, and the analyses affected by the problem repeated. There are no control limits for field replicates.

DATA REPORTING REQUIREMENTS

Report data in the units specified for the particular method. For results in which the analyte was not detected, report the results as less than the detection limit. The results of QA/QC analyses should also be provided.

CLEANING METHODS

Avoiding sample contamination requires careful cleaning of sampling equipment, sample bottles, and laboratory equipment. Some general guidelines for cleaning are presented below. Additional requirements for certain individual variables are covered in the methods sections for those variables. The procedures recommended here should be applied to sample containers and all laboratory glassware and implements that will come into direct contact with samples during collection, storage, or analysis.

Laboratory equipment should always be washed with detergent, rinsed with tap water, and rinsed 3 more times with Type 1 water (resistivity > 10 megohm-cm). Detergents must be selected with consideration of the analyses to be performed (e.g., use phosphorus-free detergent when phosphorus analysis will be performed). An ultrasonic cleaner can minimize the need for hand scrubbing. Following the water rinses, acid washing with sulfuric acid should be performed on equipment involved with nutrient analyses. After acid washing, rinse equipment completely at least 6 times with ultrapure deionized water.

If QA/QC criteria are not met, the cleaning operation should be thoroughly reviewed to determine if inadequate cleaning procedures could be causing contamination.

RECOMMENDED METHODS FOR MEASURING FLOW

USE AND LIMITATIONS

Stream flow, or discharge, is a basic hydrologic characteristic that affects morphological development of the channel, flooding behavior, bed and bank erosion, and sediment deposition. A measurement of flow must be known to estimate pollutant mass flux. Mass flux is the product of pollutant concentration and flow. Flow can either be measured manually or by continuously recording automatic instruments. The procedure discussed here will concentrate on manual methods. Automatic instruments are relatively expensive, need regular attention, and require experienced personnel to install and operate them. An organization considering using a flow recorder must be prepared to make the necessary commitment, or make an arrangement with an agency experienced in the use of flow recorders to install, and perhaps, operate them.

The common manual methods of flow measurement are as follows:

- Current meter survey
- Staff gauge
- Float survey
- Tracer survey.

The current meter survey and staff gauge techniques are emphasized here. The current meter technique involves determining flow for a cross section of the stream. Current velocity and depth data from several points along the cross section are summed to obtain total flow. A staff gauge provides an instantaneous reading of water stage (i.e., level of stream surface with respect to a known point or datum). A stage-discharge curve must be developed to estimate flow from the staff gauge reading. The curve is developed by correlating flows determined from current meter surveys with stages over a range of flow conditions. Estimating flow from timed float travel measurements can be inaccurate. Use of this method should normally be limited to low or high flow conditions when the current meter cannot be employed. Tracers include biodegradable dyes and salts that can be detected by photometric and conductometric measurements, respectively. Tracer surveys are generally less convenient and more time consuming in natural waters than current meter methods. For a discussion of additional methods, refer to U.S. Geological Survey (USGS) publications by Carter and Davidian (1968) and Buchanan and Somers (1969). The following procedures are derived from Ecology (no date) and USGS publications cited above.

FIELD PROCEDURES

Site Selection Criteria

It is important to select a representative location to establish a station for monitoring flow. Proper site selection will improve the accuracy of flow measurements at all stream discharge levels. The following criteria should be considered when establishing a discharge measurement station. However, it is rarely possible to meet all the criteria listed. Be aware of the limitations of the site selected and possible effects on measurement.

Stream Reach Criteria—The station should be located in a stream reach (i.e., longitudinal section of the stream) with the following characteristics:

- The stream should be straight for 100 m (300 ft) upstream and downstream of the staff gauge station. Otherwise, an angular flow correction must be made as specified by Buchanan and Somers (1969).
- Flow should be confined to one channel at all stages of discharge (i.e., there should be no surface or subsurface bypasses).
- Streambed should be subject to minimal scour and relatively free of plant growth.
- Streambanks should be stable, high enough to contain maximum flows, and free of brush.
- The station should be located a sufficient distance upstream so that flow from tributaries and tides does not affect stage/discharge measurements.
- All discharge stages should be measurable somewhere within the reach. It is not necessary to measure low and high flows at the identical cross section.
- The site should be readily and safely accessible.

Cross Section Criteria—The cross section in which a station is located within a stream reach should have the following characteristics:

- Streambanks should be relatively high and stable.
- The stream should be straight with parallel banks.
- Depth and velocity must meet minimum requirements of the method and instruments being used.

- The streambed should be relatively uniform with a minimal number of boulders and without heavy aquatic growth.

- Flow should be uniform and free of eddies, slack water, and excessive turbulence.

- Sites should not be located downstream of areas with rapid changes in stage or velocity.

Streamflow Measurement Using a Current Meter

Equipment—

- Measuring tape
- Depth rod
- Current meter, calibrated.

Procedure—

1. Check that the current meter is functioning properly (see *QA/QC* section below).

2. Extend a measuring tape at right angles to the direction of flow and measure the width of the cross section. Record measurements on a data sheet. Leave the tape strung across the stream.

3. Divide the width into segments using at least 20 points of measurement. If previous flow measurements have shown uniform depth and velocity, fewer points may be used. Smaller streams may also require fewer points. Measuring points should be closer together where depths or velocities are more variable. Cross sections with uniform depth and velocity can have equal spacing.

4. Record the distance from the initial starting bank and the depth.

5. Record the current velocity at each measuring point. Horizontal (from left to right bank) and vertical (top to bottom) variation of stream velocity may influence streamflow measurements. To correct for vertical differences, hydrologists have determined depths that can yield acceptable estimates of the mean velocity over a vertical profile. If the depth exceeds 0.8 m (2.5 ft), it is recommended that velocities be measured at 20 percent and 80 percent of full depth and averaged to estimate mean velocity. In the depth range 0.1-0.8 m (0.3-2.5 ft), take the velocity at 60 percent of the full depth (measured from the surface) as an estimate of the mean over the profile. Measuring velocity in water shallower than 0.1 m (0.3 ft) is difficult with conventional current meters. If much of the reach of interest is very shallow, or flow is too slow for current meter measurement, consider installing a control

section and V-notch weir.

6. Calculate flow as a summation of flows in partial areas (Figure 2) using the following equation:

$$q_n = \frac{v_a\, da\, (b_{n+1} - b_{n-1})}{2}$$

where:

b_{n-1} = distance from initial point to the preceding point [m (ft)]
b_{n+1} = distance from initial point to the following point [m (ft)]
d = mean depth of partial area n [m (ft)]
v = average current velocity in partial area n [m/sec (ft/sec)]
q = discharge in partial area n [m^3/sec (ft^3/sec)].

Streamflow Measurement Using a Staff Gauging Station

A staff gauging station is used to determine the relationship between stream stage and flow. Once this relationship is established, it is not necessary to measure flow with a rod and meter on each sampling trip. However, it is necessary to recheck the stream stage/flow relationship once each quarter and after major runoff events. The data on stream stage and flow can also be used in conjunction with precipitation data to estimate changes in stream flows that could occur when watersheds are developed. The USGS has established a network of gauging stations throughout the country. Contact the USGS for information on gauging station locations near the prospective study site.

Equipment—

- Staff gauge
- Staff gauge mounting
- Surveyor's level and rod
- Equipment for streamflow measurement using a current meter (see above).

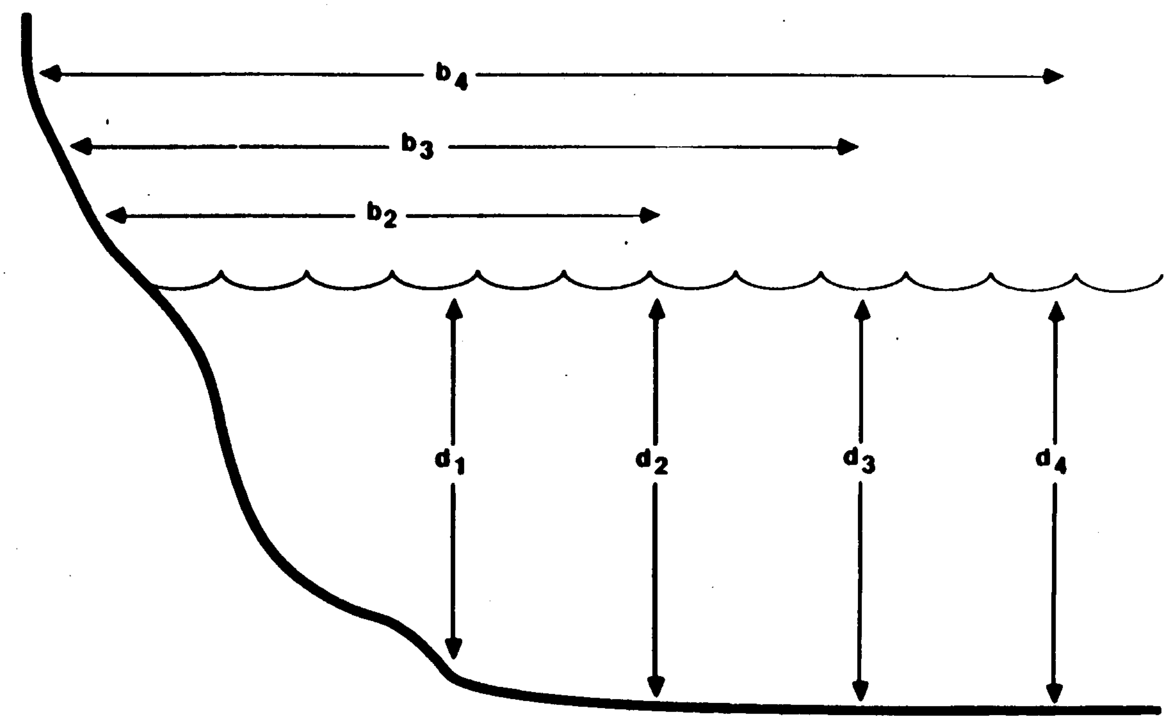

Figure 2. Diagram showing variables used in calculation of stream discharge using the current meter method

Setting Up a Staff Gauge—

1. Attach staff gauge vertically on a permanent structure (e.g., concrete piling, revetment).

2. Set the zero point of the staff gauge below the lowest level of stream flow (to prevent the occurrence of negative gauge height values).

3. Establish a datum point on the gauge, and make two or three reference marks at the same level on nearby permanent features. Use a point on the gauge that is above the highest expected gauge height to prevent flow-related erosion of the marks. The datum may also be referenced to an official surveyor's benchmark. By establishing reference elevations, the datum can be recovered if the staff gauge is destroyed.

4. Set the gauge datum to an accuracy criterion of 0.003 m (0.01 ft) and recheck it at least 2-3 times/yr.

5. Establish a stage/discharge curve (see below).

Stream stage can also be measured as the distance from the surface of the water to a permanent point above the stream. Use this measurement as the gauge height in establishing the rating curve. A bridge provides a convenient place for these measurements. The following procedures are used.

1. Make a permanent mark on the bridge to ensure that stream height is always measured from the same location.

2. Obtain stream stage by lowering a marked, weighted, measuring tape until the weight just touches the water surface. Measurement accuracy should be within 0.003 m (0.01 ft).

3. Establish a stage/discharge curve (see below).

Establishing a Stage/Discharge Curve—

1. Take streamflow measurements as described previously over a wide range of gauge heights. It will be easy to establish data points for average stream flows, but the relationship will differ for high and low stream flows. Consequently, it is important to measure during high and low stream flows so that a wide range of conditions is represented on the stage/discharge curve.

2. Note the gauge height before and after measuring flow. If wave action occurs, read height as the average of the elevations of peaks and troughs.

3. Plot calculated streamflow (i.e., discharge) on the x-axis and gauge height (i.e., stage) on the y-axis. Provide a sufficient number of points to allow a smooth curve to be drawn through the points. Curves are typically fitted by eye. As noted above, be sure the high and low ends of the curve are represented in the relationship. Examples of stage/discharge curves are depicted in Figure 3.

QA/QC PROCEDURES

There are no formal QA/QC procedures for current meter measurements. However, the meter manufacturer's guidelines for calibration should be followed.

Use of the staff gauge to measure stream flow requires that the bottom profile does not change. The bottom profile should be checked quarterly and after major runoff events. Substantial changes in the bottom profile may require redetermination of the stage/discharge relationship.

DATA REPORTING REQUIREMENTS

Report results in units of m^3/sec (ft^3/sec is also used frequently). For flows less than or equal to 10 m^3/sec, report results to the nearest 0.1 m^3/sec. For flows greater than 10 m^3/sec, report results to the nearest whole m^3/sec.

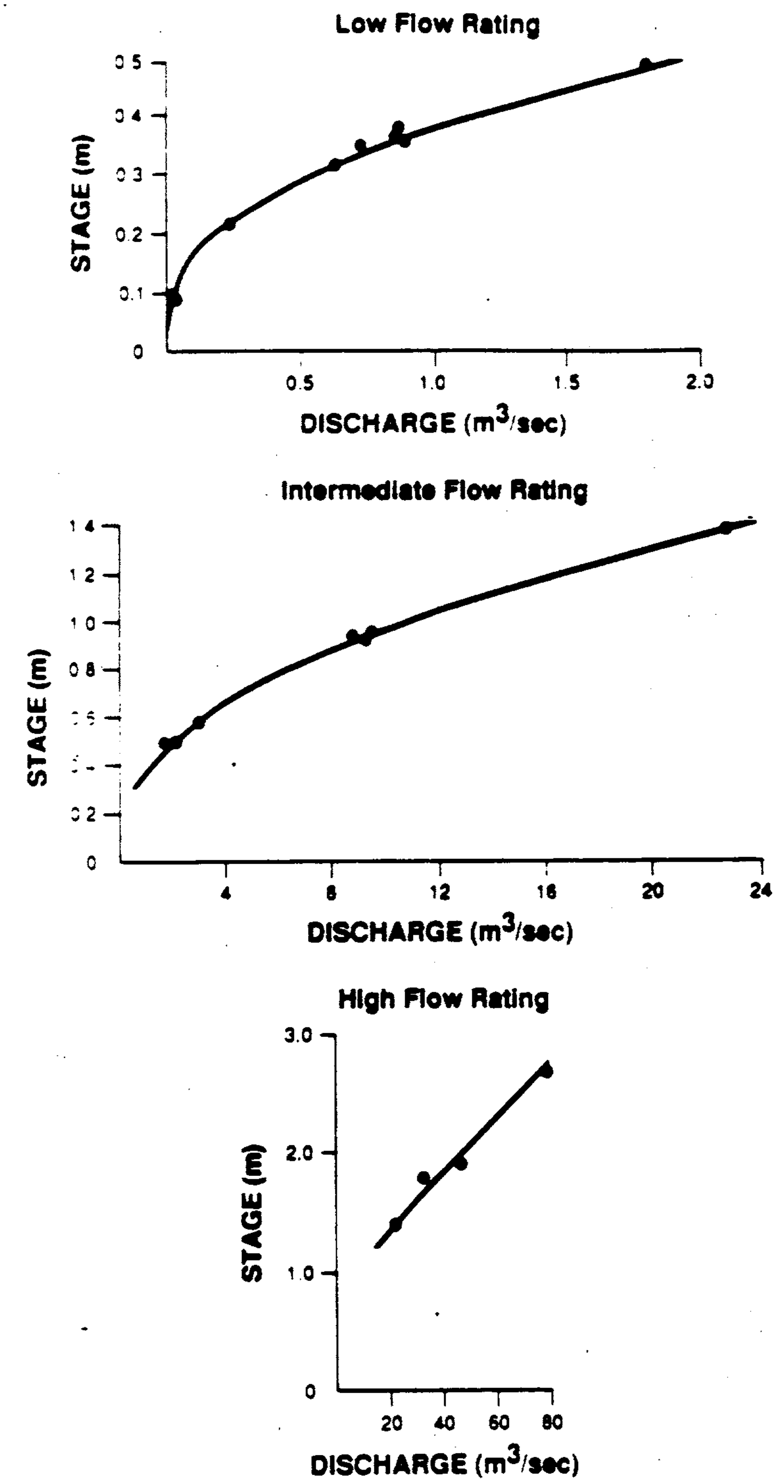

Figure 3. Examples of stream stage/discharge curves for low, intermediate, and high flow ratings

RECOMMENDED METHODS FOR MEASURING TEMPERATURE

USE AND LIMITATIONS

Temperature strongly influences the chemical and biological processes in fresh water. Organisms are adapted to live within certain temperature ranges. Hence, temperature is a key factor in determining the composition and abundance of the algal, zooplankton, macroinvertebrate, and fish communities that inhabit aquatic environments. Temperature also affects the solubility of oxygen and influences pH and conductivity. Because temperature affects density, temperature differences are the primary cause of lake stratification.

Temperature can be measured with a mercury-filled thermometer or a thermistor. Because thermometers break frequently, the thermistor is used by some organizations, even though it is more expensive. Sensitivity to the nearest 1° C is adequate for most measurements in natural waters. The requirements for laboratory measurements may be more stringent.

For more discussion of the recommended temperature measurement procedures, refer to APHA (1985) Method 212, which is equivalent to U.S. EPA (1983) Method 170.1. The following guidelines elaborate slightly on these procedures.

FIELD PROCEDURES

Collection and Measurement

Attempt to measure the temperature at a station at the same time of day on each sampling trip. Measure temperature directly in the water if possible. Otherwise, equilibrate a bottle to the water temperature, collect a sample at least 1 L in volume, and measure the temperature immediately. Allow the thermometer to come to equilibrium before recording the reading.

LABORATORY PROCEDURES

None.

QA/QC PROCEDURES

Refer to Table 5 for the recommended calibration technique.

DATA REPORTING REQUIREMENTS

Report results to the nearest 0.1 or 1.0° C, depending on need and accuracy of the measurement. Also report the time of day.

RECOMMENDED METHODS FOR MEASURING CONDUCTIVITY

USE AND LIMITATIONS

Conductivity is a measure of the ability of water to conduct an electric current. Because temperature affects the velocity of ion movements, conductivity is based on a specific reference temperature.

The movement of positive and negative ions in a solution creates an electrical current. The relationship between conductivity and ion concentration varies with the type and relative amount of ions present. For example, at relatively low ion concentrations the ions move independently, and the relationship between conductivity and ion concentration is nearly linear.

Conductivity is a general indicator of the combined concentration of ions, and not a measure of any particular substance. Conductivity is related to water quality for many soluble pollutants (e.g., nutrients). Therefore, conductivity can be used to detect the existence of a contamination problem, but cannot be used to identify the specific problem.

Conductivity is measured using a conductivity cell and meter. When the cell's two electrodes are inserted into a water sample, the meter emits an electrical signal and measures the ease with which the electrical current is conducted through the sample. Conductivity is the inverse of resistance, and its unit (mho) is a transposition of the unit (ohm) for resistance. Recently, this unit was termed the siemen (S) in the SI system of units. Because the measurement is usually performed with electrodes that are 1 cm apart, conductivity is usually reported as µmho/cm [or microsiemens/cm (µS/cm) in the SI system].

The most common reference temperature for conductivity is 25° C. During analysis, the temperature of the sample must be taken. Some meters automatically correct the temperature. If the meter does not automatically correct the temperature, then the temperature correction must be calculated (see *Calculations* section below). The use of a common reference temperature allows comparison of data from samples that may have been analyzed at different temperatures.

For a full discussion of the recommended conductivity measurement procedure refer to either APHA (1985) Method 205, or U.S. EPA (1983) Method 120. The following guidelines elaborate on these procedures, and include a specific recommendation for a two-point rather than a single-point initial calibration.

FIELD PROCEDURES

Sample Collection

Samples can be collected in polyethylene, polypropylene, fluoropolymer [e.g., polytetrafluoroethylene (Teflon™)], or glass containers. A minimum sample volume of 100 mL is recommended.

Sample Processing and Storage

If analysis is not completed within 24 h of sample collection, the sample should be filtered through 0.45-µm filter, and the filtrate should be stored at 4° C in the dark. A sample can be stored up to 28 days after it has been filtered.

LABORATORY PROCEDURES

Equipment Selection and Standard Preparation

- Conductivity meter. A temperature-compensating meter is preferred.
- Conductivity cell.
- Thermometer or digital probe, capable of being read to 0.1° C and covering the range in which the conductivity measurements will be made.
- Standard potassium chloride (KCl) solutions representing the same conductivity range as found in the samples. Prepare a standard 0.0100 N KCl reference solution, which has a conductivity of 1,413 µmhos/cm at 25° C, by dissolving 0.7456 grams predried (2 hours at 105° C) KCl in 1-liter low-conductivity (<1 µmho/cm) distilled water. Dilute with low-conductivity distilled water to make up standard solutions in the desired range.

Equipment Preparation

1. Determine the conductivity cell constant frequently (i.e., according to the manufacturer's recommendations) before analyzing samples. Rinse conductivity cell at least 3 times with standard KCl reference solution. Adjust temperature of a sample of the standard KCl reference solution to 25.0 ± 0.1° C and measure its conductivity. Determine the cell constant by dividing the conductivity of the standard KCl reference solution by this measurement. The constant should either be 1.0, or very close to it.

2. Calibrate the conductivity cell in the laboratory with a standard KCl reference solution at 25.0 ± 0.1° C in the expected conductivity range of the samples. Rinse the cell with the standard KCl reference solution prior to taking the measurement.

3. Check calibration once for each batch of samples, and whenever the meter is setup in the field. Rinse at least 3 times with the standard KCl reference solution prior to taking a measurement. The conductivity of the standard should be within the expected conductivity range of the samples.

Sample Preparation

1. If a nontemperature-compensating meter is used, adjust sample temperature to 25° C.

2. Determine sample temperature within 0.5° C.

Sample Analysis

1. Rinse cell at least 3 times with sample.

2. Measure the conductivity according to the instructions provided with the meter. If the sample temperature is not 25° C, then either compensate with the meter adjustment (if the meter has that feature), or correct to 25° C as shown in the *Calculations* section below.

3. Rinse the cell with distilled water after use. Keep electrodes immersed in Type 1 water (resistivity > 10 megohm-cm) afte use.

Calculations

Correct for cell constant and temperature, if necessary, as follows:

Conductivity = measured conductivity x cell constant x temperature correction factor

Temperature correction factors are given in Table 6.

TABLE 6. TEMPERATURE CORRECTION FACTORS FOR CONDUCTIVITY DETERMINATION

Temperature (° C)	Correction Factor[a]
2	1.70
3	1.65
4	1.61
5	1.57
6	1.53
7	1.49
8	1.45
9	1.41
10	1.37
11	1.34
12	1.31
13	1.28
14	1.25
15	1.22
16	1.19
17	1.16
18	1.14
19	1.12
20	1.10
21	1.08
22	1.06
23	1.04
24	1.02
25	1.00

[a] Correction factors are used only when the conductivity meter does not compensate for temperature.

QA/QC PROCEDURES

Conductivity QA/QC depends upon regularly checking the cell constant as described in the previous section entitled *Equipment Preparation*. In addition, 5 to 10 percent of the samples should be randomly selected for duplicate field collection, and 5 to 10 percent of the samples should be randomly selected for duplicate laboratory analysis. Based on data reported by U.S. EPA (1983) and APHA (1985), the relative precision of this QA/QC method is 7.8-8.6 percent, and the relative accuracy is 1.9-9.4 percent (APHA 1985).

DATA REPORTING REQUIREMENTS

Results should be reported to the nearest 1 µmho/cm (or 1 µS/cm) at 25° C. The actual temperature at which the measurement was made should be reported, as well as the results of all QA/QC analyses.

RECOMMENDED METHODS FOR MEASURING DISSOLVED OXYGEN

USE AND LIMITATIONS

Most metabolic processes require oxygen. Oxygen is depleted from aquatic media by respiration of aquatic organisms, decomposers (i.e., bacteria and fungi) during biodegradation of organic matter, and chemical processes in which oxygen reacts with organic and inorganic substances. Photosynthesis and interactions between water and the atmosphere can increase the oxygen concentration in the water.

Solubility of gases is generally higher in colder water. Therefore, DO concentrations should be interpreted in terms of water temperature. This relationship is expressed as percent saturation. This value is obtained by expressing, as a percentage, the ratio of the observed DO concentration in a sample to the theoretical DO saturation concentration of water at the same temperature. Supersaturation (i.e., >100 percent saturation) tends to occur after several daylight hours (i.e., plants have been producing oxygen as a byproduct of photosynthesis). DO concentrations recorded during the early morning hours are often below 100 percent saturation because the production of oxygen by photosynthesis ceases in the dark, while the consumption of oxygen by respiration continues. Therefore, organisms would be most stressed by low DO concentrations during the early morning. Studies of low DO should include sampling during the period from predawn to early morning, and sampling should be avoided during the late afternoon, when photosynthetic activity is generally highest.

Salmonid fish and their preferred invertebrate foods inhabit water with consistently high DO concentrations (i.e., above 5 mg/L). Other fishes (e.g., bass, perch) have less restrictive oxygen requirements, but DO concentrations of at least 4 mg/L are generally necessary for most species in this group. As DO declines to very low levels, less desirable organisms (e.g., dipteran larvae) tend to predominate in the community.

DO can be measured by either chemical titration (azide-modified Winkler method) or membrane electrode method. The former is recommended for general field monitoring work because of its ease and low cost, and because of the difficulty in maintaining meter calibration. A well-calibrated oxygen meter membrane electrode system is preferred for obtaining a depth-profile of DO in a lake or deep river. Sampling for DO measurements requires particular care, since any contact between the sample and the air will modify the results. If percent saturation is to be determined, then the water temperature must be measured at the same time and location.

The recommended procedures for the azide-modified Winkler method are APHA (1985) Method 421B, and U.S. EPA (1983) Method 360.2. While these methods are equivalent, they differ in some instructions for preparing and adding reagents. Therefore, the technician must choose a method and follow it consistently. The APHA (1985) Method 421F, and U.S. EPA (1983) Method 360.1 are recommended methods for the membrane electrode technique. These methods are also equivalent. See these references for information concerning problems with interfering substances (e.g., iron). The following guidelines elaborate on both sets of recommended methods.

FIELD PROCEDURES

Azide-Modified Winkler Method

Sample Collection—Samples must be collected in 300 mL glass BOD bottles. In deeper waters, a Van Dorn or other appropriate sampler (e.g., see APHA 1985) should be used to obtain the sample. In shallow waters (i.e., where a water-bottle sampler cannot be used), use a hand pump or a bucket with a clamped drain tube installed at the bottom. Insert the outlet tube of the sampling apparatus to the bottom of the BOD bottle. Overflow the bottle by two or three volumes. Take care to prevent turbulence and bubble formation. Fill the bottle to the rim and insert stopper, being sure that no air becomes trapped in the bottle. The temperature of the water source should be read during sampling.

Sample Processing and Storage—Samples with no iodine demand (generally the case in fresh water) may be stored up to 8 h without change after adding manganous sulfate solution, alkali-iodide-azide solution, and concentrated sulfuric acid, followed by shaking. Pour some distilled water around the glass stopper and cover with aluminum foil held in place with a rubber band. Store in the dark at the temperature of the water source, or at 10-20° C. Complete the analysis as soon as possible (i.e., within 8 h).

Membrane Electrode Method

Equipment Selection—Select equipment as discussed by APHA (1985) Method 421F.2, or U.S. EPA (1983) Method 360.1.5.1.

Equipment Preparation—Calibrate the meter prior to each series of measurements, or whenever the meter is moved or turned off. Calibrate with a sample of known DO concentration (determined by analysis of distilled water according to the azide-modified Winkler method), as well as with a sample with zero DO. To bring DO to zero, add excess sodium sulfite (Na_2SO_3) and a trace of cobalt chloride ($CoCl_2$). Follow the meter manufacturer's calibration procedure exactly.

When membrane function deteriorates it should be changed to avoid contamination of the sensing element. Air bubbles should not be trapped under the membrane.

Sample Analysis—Follow all instructions of the meter manufacturer exactly. If erratic responses occur, then stir the sample or provide sufficient sample flow across the membrane surface.

LABORATORY PROCEDURES

Azide-Modified Winkler Method

Equipment Selection and Reagent Preparation—Select equipment and prepare reagents according to APHA (1985) Method 421B.2, or U.S. EPA (1983) Methods 360.2.5 and 360.2.6.

Equipment Preparation—Run one calibration sample per batch of samples. In most DO studies of well oxygenated waters, the calibration sample is prepared by bubbling air into distilled water to obtain a sample with 100 percent DO saturation. If the samples are expected to contain low DO concentrations, then a zero DO calibration sample (prepared as discussed above) can be used.

Sample Analysis—Analyze according to APHA (1985) Method 421B.3, or U.S. EPA (1983) Method 360.2.7.

Calculations—Results can be expressed as mg/L (along with the corresponding values of temperature, pressure, and salinity), or as percent saturation. Results can be expressed as percent saturation as follows:

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click here to view equation.

Obtain DO solubility for the temperature, pressure, and salinity conditions at which the sample was taken [see Table 421.I APHA (1985), or U.S. EPA (1983) Method 360.2.8.4].

Membrane Electrode Method

Except for the calibration and QA/QC procedures, which use the azide-modified Winkler procedures outlined above, no laboratory procedures are required.

QA/QC PROCEDURES

For the azide-modified Winkler method, run one calibration sample per batch of samples. Prepare the calibration sample by bubbling air into distilled water. Standardize the sodium thiosulfate titrant both before and after a set of samples is analyzed, and adjust the dissolved oxygen measurements accordingly. Phenylarsine oxide (PAO) may be used instead of sodium thiosulfate and can be purchased, already standardized, from commercial sources. Randomly select 5-10 percent of the samples for duplicate field collection, and 5-10 percent of the samples for duplicate laboratory analysis. For the membrane-electrode method, calibrate the meter prior to each series of measurements, and whenever the meter is moved or turned off. The reported precision of the azide-modified Winkler method is 0.02-0.06 mg/L. The membrane electrode method has a reported precision of 0.05 mg/L, and a reported accuracy of 0.1 mg/L (APHA 1985).

DATA REPORTING REQUIREMENTS

Report results to the nearest 0.1 mg/L. Include the results of all QA/QC analyses in the data report.

RECOMMENDED METHODS FOR MEASURING pH

USE AND LIMITATIONS

The pH of a solution is a measure of the hydrogen ion activity. By definition, pH is the negative base-10 logarithm of the hydrogen ion concentration in moles/L. A pH of 7 represents equality of hydrogen and hydroxyl ion concentrations, and therefore neutrality, while pH <7 represents an acidic condition (predominance of hydrogen ions) and pH >7 (maximum 14) represents a basic condition (predominance of hydroxyl ions). Because of the logarithmic scale, a change of one pH unit represents a tenfold change in the hydrogen ion concentration.

Most aquatic animals have pH preferences from 6 to 9. This range is usually maintained by carbonate and bicarbonate buffering of naturally acidic precipitation. Due to carbon dioxide solubility, rain that has been affected by no other dissolved gases or ions has a theoretical pH of 5.65. The presence of acidic or alkaline compounds can lower or raise the pH of a water body, respectively.

The pH of water profoundly affects the chemical processes that may occur within it. The chemical speciation of important water quality constituents such as nutrients and metals is chiefly determined by pH. Metals tend to be more soluble, and therefore more available to organisms at acidic pH values than at basic pH values. Hence, toxicity can be increased by acidification.

An electronic meter is used to measure pH. This measurement is accurate only in a fresh sample. Rapid pH changes occur because of gas diffusion, biological activity, and chemical reaction. Therefore, pH measurements must be performed in the field, immediately after sampling.

The recommended procedures for measurement of pH are APHA (1985) Method 423 and U.S. EPA (1983) Method 150.1. These procedures cannot be used for seawater or brackish water. These procedures are elaborated slightly in the following guidelines.

FIELD PROCEDURES

Sample Collection

Samples can be collected in polyethylene, polypropylene, fluoropolymer, or glass containers. The same container can be used for samples intended for pH measurement, and for samples intended for other variables that will be chilled for transport to the laboratory. A minimum sample size of 25 mL is needed for a pH measurement. Additional water should be collected to rinse the electrodes several times, especially in low-alkalinity waters.

Sample Processing and Storage

Samples must be analyzed for pH in the field, immediately after collection.

Equipment and Buffer Selection

Select equipment and buffer solutions as described for APHA (1985) Methods 423.2 and 423.3 or U.S. EPA (1983) Methods 150.1.5 and 150.1.6. It is recommended that three buffer solutions be available for calibration (one at or near neutral pH, one at acidic pH $\leq$4.0, and one at alkaline pH $\geq$10.0).

Equipment Preparation

1. Follow the pH meter manufacturer's instructions for storage and preparation of the electrodes.

2. Remove electrodes from storage solution, rinse with distilled water, and blot dry with a soft tissue.

3. In the field, standardize the meter every 3 h using the aforementioned buffer solutions and following the manufacturer's instructions. Standardize with the neutral buffer, and either the acidic or the alkaline buffer, depending on the pH range expected for the samples. For each buffer solution, bring the temperature as close as possible to the sample temperature. Place electrodes in solution for at least 1 min. Wait until the reading becomes drift-free (<0.1 pH unit). Between samples, rinse electrodes with distilled water and blot dry.

Sample Analysis

Analyze according to APHA (1985) Method 423.4, or U.S. EPA (1983) Method 150.1.8. Handle the electrodes and read the pH of samples in the same manner as described for equipment preparation above. The electrodes should be rinsed with the sample several times, especially when analyzing low-alkalinity waters.

QA/QC PROCEDURES

QA/QC for pH depends on regularly standardizing the meter, as described above. In addition, randomly select 5-10 percent of the samples for duplicate field collection, and 5-10 percent of the samples for duplicate analysis. This method has a reported precision of 0.13 pH unit, and a reported accuracy of 0.1 pH unit (APHA 1985).

DATA REPORTING REQUIREMENTS

Report results to the nearest 0.1 pH unit. The pH and temperature of the buffers used for calibration should also be reported. Include the results of all QA/QC analyses in the data report.

RECOMMENDED METHODS FOR MEASURING ALKALINITY

USE AND LIMITATIONS

The alkalinity of water is a measure of its acid-neutralizing capacity. Alkalinity represents the amount of acid required per unit volume to lower the pH to a designated point in the acid region (the titration endpoint). The primary importance of alkalinity for natural waters is to quantify the ability of water to buffer acids that may enter (e.g., from spills or acid precipitation). In the Puget Sound region, fresh waters are typically relatively low in alkalinity because of the low solubility of parent geological materials.

Alkalinity is an aggregate water quality variable, and cannot be interpreted in terms of specific substances without knowledge of the specific chemical composition of the sample. Although alkalinity is the sum of all titratable bases, it is primarily a function of carbonate (CO_3^-), bicarbonate (HCO_3^-), and hydroxide (OH^-) content. It is defined mathematically by the following charge balance equation:

$$\text{Alkalinity} = [HCO_3^-] + 2[CO_3^{--}] + [OH^-] - [H^+]$$

For natural waters in the pH ranges from 6 to 8.5, $[CO_3^{--}]$, $[OH^-]$ and $[H^+]$ are negligible, and alkalinity is essentially equal to $[HCO_3^-]$.

Alkalinity is determined by titrating the sample with strong acid to pH 4.2 to 4.5, depending on the amount of alkalinity. Recently published methods [e.g., APHA (1985)] recommend an endpoint at, or near, pH 4.2 for low-alkalinity samples.

In practice, alkalinity is frequently reported in units of mg/L as Ca/CO_3 that would produce an alkalinity value equivalent to that measured in the sample (e.g., 50 mg/L as $CaCO_3$). The recommended calculations performed after the analysis produce results in these units.

FIELD PROCEDURES

Sample Collection

Samples can be collected in polyethylene, polypropylene, fluoropolymer, or glass containers. The same container can be used for samples intended for analysis of alkalinity and samples intended for analysis of other variables with compatible preservation. A minimum sample size of 100 mL is recommended.

Sample Processing and Storage

Samples should be stored at 4° C in the dark. It is preferable to perform the analysis within 24 h, but samples can be stored up to 14 days in the above condition.

LABORATORY PROCEDURES

The recommended procedures for alkalinity measurement are APHA (1985) Method 403, and U.S. EPA (1983) Method 310.1. The following guidelines add some detail to these procedures, particularly with regard to analysis of low-alkalinity waters.

Equipment Selection and Reagent Preparation

Select equipment and prepare reagents according to APHA (1985) Methods 403.2 and 403.3, or U.S. EPA (1983) Methods 310.1.4 and 310.1.5.

Sample Analysis

Analyze samples as specified by APHA (1985) Method 403.4, or by U.S. EPA (1983) Method 310.1.6. If alkalinity is <20 mg/L as $CaCO_3$, which is common in fresh waters of the Puget Sound region, then follow the procedures designed for low-alkalinity waters [APHA (1985) 403.4d, or U.S. EPA (1983) 310.1.6.3].

Calculations

Calculate alkalinity in units of mg/L as $CaCO_3$ according to APHA (1985) Method 403.6 or U.S. EPA (1983) 310.1.7.

QA/QC PROCEDURES

Regularly standardize the pH meter, as specified in the pH section. With each batch of samples, standardize the acid titrant, or run U.S. EPA control solutions of known concentrations. In addition, randomly select 5-10 percent of the samples for duplicate field collection, and 5-10 percent of the samples for duplicate laboratory analysis. According to data reported by U.S. EPA (1983), the method precision ranges from 1.14 to 5.36 mg/L as $CaCO_3$, and the accuracy ranges from -9.3 to +2.0 mg/L as $CaCO_3$ over a concentration range of 8-119 mg/L as $CaCO_3$.

DATA REPORTING REQUIREMENTS

Report results to the nearest 1 mg/L as $CaCO_3$. Include results of all QA/QC analyses in the data report.

RECOMMENDED METHODS FOR MEASURING TOTAL HARDNESS

USE AND LIMITATIONS

Originally, the term total hardness referred to the soap-neutralizing power of water. Because soap is alkaline, higher concentrations of other alkaline substances retard the neutralization of soap and cause it to form an insoluble precipitate. Divalent metallic cations (+2 charge) are capable of reacting with soap to form precipitates. The principal hardness-causing divalent cations are calcium and magnesium, with strontium, ferrous iron and manganese of minor importance.

The hardness of water is derived largely from its contact with soil and rock. Carbon dioxide in chemical equilibrium with carbonic acid gives water the ability to dissolve minerals. In general, hard waters exist in areas where the topsoil is thick and limestone ($CaCO_3$) formations are present. Soft waters (i.e., waters with low hardness) exist where there is little topsoil or limestone.

Hardness creates difficulties in laundering and promotes accumulation of scale in hot water pipes and other heated equipment. Calcium in water may also promote kidney stone formation. However, substances creating hardness are antagonistic to some potentially toxic heavy metals.

Current practice is to define total hardness as the sum of calcium and magnesium concentrations. Total hardness is normally expressed in terms of the mg/L of $CaCO_3$ that would have the effect that is equivalent to the measured total hardness.

The recommended technique for total hardness measurement is the disodium ethylene-diaminetetraacetate dihydrate titrimetric (EDTA) method. The full procedures for this method are provided by APHA (1985) Method 314B and U.S. EPA (1983) Method 130.2. While these methods are equivalent, they differ in some instructions for preparing and adding reagents. Therefore, the analyst must follow one or the other consistently. The following guidelines elaborate on these methods.

FIELD PROCEDURES

Sample Collection

Samples can be collected in polyethylene, polypropylene, fluoropolymer, or glass containers. The same container can be used for samples intended for analysis of total hardness and for samples intended for analysis of other variables with compatible preservation. A minimum sample size of 100 mL is recommended.

Sample Processing and Storage

Samples should be acidified with nitric acid to pH <2. Acidified samples can be held up to 6 months.

LABORATORY PROCEDURES

Equipment Selection and Reagent Preparation

Select equipment and prepare reagents according to APHA (1985) Method 314B.2 or U.S. EPA (1983) Methods 130.2.5 and 130.2.6.

Sample Analysis

Analyze according to APHA (1985) Method 314B.3 or U.S. EPA (1983) Method 130.2.7.

When ions in the water sample interfere with endpoint determination, it may occasionally be necessary to use sodium cyanide as an inhibitor when analyzing hardness. This problem occurs rarely for the natural fresh waters of the Puget Sound region. Because cyanides are poisonous, they should be used with caution and disposed of properly. The sodium cyanide should be used only in a ventilation hood. Acidification of cyanide solutions can liberate lethal hydrogen cyanide gas. Although APHA (1985) and U.S. EPA (1983) suggest that used cyanide can be flushed down the drain with large volumes of water (provided that acids are not present in the drain), this method of disposal is not environmentally sound and is not recommended in the PSEP protocols. The current practice for cyanide disposal at the U.S. EPA Manchester laboratory is to retain the used cyanide in a bottle that is stored in a designed hazardous waste area. When the bottle is full, the cyanide concentration is determined, and the bottle is disposed of by an approved hazardous waste contractor (Davis, P., 11 October 1988, personal communication).

Calculations

Calculate total hardness as mg $CaCO_3$/L according to APHA (1985) Method 314B.4 or U.S. EPA (1983) Method 130.2.8.

QA/QC PROCEDURES

With each batch of samples, standardize the EDTA titrant, or run U.S. EPA control solutions of known concentrations. Also, run one blank with each batch. If interference caused by high heavy metal concentrations is suspected, run one spiked sample with each batch. In addition, randomly select 5-10 percent of the samples for duplicate field collection and 5-10 percent of the samples for duplicate laboratory analysis. According to data reported by APHA (1985), the relative precision of the method is 2.9 percent, and its relative accuracy is 0.8 percent.

DATA REPORTING REQUIREMENTS

Report results to the nearest 1 mg/L as $CaCO_3$ and report the use of any inhibitor. Include the results of all QA/QC analyses in the data report.

RECOMMENDED METHODS FOR MEASURING TOTAL SUSPENDED SOLIDS

USE AND LIMITATIONS

Total suspended solids (TSS) is a direct measure of the concentration of particulate matter in flowing or standing water. Particles may be organic (e.g., detritus, algae) or inorganic (e.g., eroded mineral soil components). The significance of suspended solids on water quality is manifested in several ways. Suspended solids can reduce light penetration and damage the tissues and clog the respiratory apparatus of aquatic organisms. Upon settling, excessive solids degrade bottom habitat, especially by filling the gravels that are favorable for invertebrate habitat and the spawning and rearing of fish. Solids also transport other pollutants, including oxygen demanding substances, nutrients, metals, xenobiotic organic compounds, and pathogenic microorganisms.

The concentration of TSS is measured gravimetrically as the mass of particles retained on a filter per unit volume of water filtered. Another commonly used term for TSS is nonfilterable residue.

The recommended analytical procedures for TSS are APHA (1985) Method 209C and U.S. EPA (1983) Method 160.2. The following guidelines elaborate on these procedures, particularly with regard to sample collection.

FIELD PROCEDURES

Sample Collection

Samples can be collected in polyethylene, polypropylene, fluoropolymer, or glass containers. Because there typically is a gradient of particle concentrations over depth in streams, sampling over a depth profile is preferred over sampling at a single depth. However, because such sampling is burdensome, a single sample is usually collected. Collection at a point representing the average velocity in the depth profile (for streams that are deep enough for this to be a concern) is superior to sampling at mid-depth. The average current velocity generally occurs at 60 percent of full depth from the surface. Another practice often followed during wet weather sampling is to collect at 30 percent (from the surface) of the winter baseflow depth. The same container can be used for samples to be analyzed for TSS and for samples to be analyzed for other variables with compatible sampling requirements and preservation. A minimum sample size of 1 L is recommended for the least concentrated samples, although 100-250 mL is sufficient for most samples.

Sample Processing and Storage

Samples should be stored in the dark at 4° C. In this condition, samples can be held as long as 7 days.

LABORATORY PROCEDURES

Equipment Selection

Select equipment as designated by APHA (1985) Method 209C.2 or U.S. EPA (1983) Method 160.2.6.

Equipment Preparation

Prepare equipment as specified by APHA (1985) Method 209C.3a or U.S. EPA (1983) Method 160.2.7.1.

Sample Preparation

Select filter and sample sizes as specified by APHA (1985) Method 209C.3b or U.S. EPA (1983) Method 160.2.7.2.

Sample Analysis

Analyze according to APHA (1985) Method 209C.3c or U.S. EPA (1983) Method 160.2.7.3-6.

Calculations

Calculate TSS in mg/L according to APHA (1985) Method 209C.4 or U.S. EPA (1983) Method 160.2.8.

QA/QC PROCEDURES

Check balance calibration monthly and oven temperature daily. Balances should have annual preventative maintenance checks. Run at least one EPA or commercial control suspension of known concentration per set of 20 samples. In addition, randomly select 5-10 percent of the samples for duplicate field collection and 5-10 percent of the samples for duplicate laboratory analysis. The method has a reported precision of 2.8 mg/L (APHA 1985).

DATA REPORTING REQUIREMENTS

Report results to the nearest 1 mg/L. Include the results of all QA/QC analyses in the data report.

RECOMMENDED METHODS FOR MEASURING TURBIDITY

USE AND LIMITATIONS

Turbidity is the optical property of water that causes light to be scattered and absorbed, rather than to be transmitted. Turbidity is caused by suspended particles such as clay, silt, colloidal organic and inorganic matter, plankton, and other microorganisms. These particles affect light penetration in water.

Turbidity is determined relative to a standard reference suspension using an instrument, called a nephelometer, that measures light scattering. Therefore, the measurement does not absolutely represent particulate concentration. Correlation of turbidity with the concentration of suspended material is possible but difficult. Particles of different sizes, shapes, and refractive indices have different light-scattering properties. Useful correlations usually can be made only in specific cases, when the nature of the particles remains constant while their concentration varies.

The reference suspension for turbidity measurement is formazin, a polymer with particles of particular size and light-scattering characteristics. Readings are reported in a scale relative to the standard suspension. The units are termed nephelometric turbidity units (NTU). The turbidity of the reference formazin suspension is defined as 40 NTU.

The recommended procedures for turbidity analysis are APHA (1985) Method 214A and U.S. EPA (1983) Method 180.1. The following guidelines elaborate on these procedures, particularly with regard to sample collection.

FIELD PROCEDURES

Sample Collection

Samples can be collected in polyethylene, polypropylene, fluoropolymer, or glass containers. A minimum sample volume of 100 mL is recommended. In a stream, the same considerations apply to selecting the turbidity sampling depth as apply to selecting the TSS sampling depth. Samples should be collected at 60 percent of full depth from the surface. During wet weather, samples can be collected at 30 percent of the winter baseflow depth from the surface.

Sample Processing and Storage

Samples should be stored in the dark at 4° C and analyzed within 24 h of collection, if possible. Samples may be held for up to 48 h in this condition.

LABORATORY PROCEDURES

Equipment Selection and Standard Preparation

Select equipment and prepare standard suspensions as designated by APHA (1985) Methods 214A.2 and 214A.3 or U.S. EPA (1983) Methods 180.1.5 and 180.1.6.

Equipment Preparation

If precalibrated scales are supplied with the instrument, check their accuracy using appropriate standards. If precalibrated scales are not supplied, prepare calibration curves for each turbidity range. Run at least one standard every time the range is changed.

Sample Analysis

Analyze according to APHA (1985) Method 214A.4 or U.S. EPA (1983) Method 180.1.7. Samples with turbidities >40 NTU should be diluted to a turbidity between 30 and 40 NTU for analysis. Turbidity-free water should be used for sample dilution.

Calculations

Calculate as specified by APHA (1985) Method 214A.5. For turbidities <40 NTU the data can be read directly from the turbidometer or from an appropriate calibration curve.

QA/QC PROCEDURES

QA/QC procedures for turbidity depend on recalibrating the turbidimeter with every range change. In addition, randomly select 5-10 percent of the samples for duplicate field collection and 5-10 percent of the samples for duplicate analysis. According to data reported by U.S. EPA (1983), the method precision ranges from 0.60 to 4.7 NTU over a turbidity range of 26 to 180 NTU.

DATA REPORTING REQUIREMENTS

Report results as designated for various turbidity ranges by APHA (1985) Method 214A.2 or U.S. EPA (1983) Method 180.1.8. Include the results of calibration analyses in the data report.

RECOMMENDED METHODS FOR MEASURING AMMONIA-NITROGEN

USE AND LIMITATIONS

Ammonia is a component of fertilizers, sewage effluents, and manure. It is an inorganic form of nitrogen that is highly soluble in water. Ammonia can be released by deamination of organic nitrogen-containing compounds and hydrolysis of urea. Depending upon pH, ammonia may be present as the aqueous gas (NH_3) or the ammonium ion (NH_4^+). At usual natural water pH, the ammonium ion predominates. Under aerobic conditions, ammonia can be converted to nitrite (NO_2^-) and then nitrate (NO_3^-) through the bacterial process of nitrification.

Ammonia is a key nitrogen-containing nutrient for algae and aquatic plants and is readily absorbed from water by these organisms. Therefore, increasing the ammonia concentration can stimulate eutrophication in waters in which algal growth is nitrogen-limited. Ammonia also influences oxygen demand because nitrification consumes dissolved oxygen. Excessive concentrations of the non-ionized (NH_3) form are toxic to aquatic organisms.

Ammonia (ammonia plus ammonium ion) is measured as nitrogen and is generally reported as ammonia-nitrogen. The most common analysis is performed by the automated phenate method using an autoanalyzer, although the manual phenate method is also recommended. APHA (1985) Methods 417C and 417G contain procedures for the manual and automated methods, respectively. The U.S. EPA (1983) supplies only an automated procedure (Method 350.1), which is equivalent to APHA (1985) Method 417G. These methods offer the low detection limits often necessary to measure ammonia-nitrogen in natural fresh waters of the Puget Sound region.

FIELD PROCEDURES

Sample Collection

Samples can be collected in polyethylene, polypropylene, fluoropolymer, or glass containers. A minimum sample volume of 125 mL is recommended. Samples for analysis of ammonia-nitrogen can be collected in the same containers as samples intended for analysis of other variables that are compatibly handled and preserved (generally including nitrate+nitrite-nitrogen and total phosphorus).

Sample Processing and Storage

Samples should be acidified with sulfuric acid to pH <2 and stored in the dark at 4° C. They should be analyzed within 7 days, if possible, but may be held up to 28 days in this condition.

LABORATORY PROCEDURES

To avoid contaminating water samples being stored and analyzed for ammonia-nitrogen, do not use or produce ammonia in the storage and laboratory areas.

Automated Method

Equipment Selection and Reagent Preparation—Select equipment and prepare reagents as designated by APHA (1985) Methods 417G.2 and 417G.3 or U.S. EPA (1983) Methods 350.1.5 and 350.1.6. Although these procedures are based on Technicon AutoAnalyzer™ technology, other acceptable autoanalyzers (e.g., Alpkem™) exist. Either segmented (i.e., autoanalyzer) or non-segmented (i.e., flow injection analysis) continuous flow analyzers can be used.

Equipment Preparation—Prepare the autoanalyzer as specified by APHA (1985) Methods 417G.2 and 417G.4, U.S. EPA (1983) Methods 350.1.5 and 350.1.7, or equivalent manufacturers instructions. Run a calibration curve with a blank and standards at 0.2, 0.5, and 1.0 times the expected highest concentration in a sample. The entire range of sample concentrations must be included in the calibration curve.

Sample Preparation—Prepare samples and standards as specified by APHA (1985) Method 417G.4a or U.S. EPA (1983) Method 350.1.7.1.

Sample Analysis—Analyze according to APHA (1985) Method 417G.4 or U.S. EPA (1983) Method 350.1.7.

Calculations—Prepare a standard curve by plotting peak height of standards against ammonia-nitrogen concentrations in the standards (or compute a linear regression equation for ammonia-nitrogen concentration as a function of peak height). Calculate sample ammonia-nitrogen concentrations from the standard curve or regression equation.

Manual Method

Equipment Selection and Reagent Preparation—Select equipment and prepare reagents as designated by APHA (1985) Methods 417C.2 and 417C.3.

Equipment Preparation—Run calibration curve with a blank and standards at 0.2, 0.35, 0.50, 0.75, and 1.0 times the expected highest concentration in a sample. The entire range of sample concentrations must be included in this calibration curve. A reagent blank (i.e., a sample consisting of distilled water to which all reagents have been added) should be used to obtain the zero rating on the spectrophotometer prior to the analysis of standards and samples.

Sample Preparation—Prepare standards as specified by APHA (1985) Method 417C.4b.

Sample Analysis—Analyze according to APHA (1985) Method 417C.4.

Calculations—Prepare a standard curve by plotting absorbances of standards against ammonia-nitrogen concentrations in the standards (or calculate a linear regression equation for ammonia-nitrogen concentration as a function of absorbance). Calculate sample ammonia-nitrogen concentrations from the standard curve or regression equation.

QA/QC PROCEDURES

Run control samples at 20 percent and 90 percent of the upper limit of the expected concentration range with each batch of 10 to 20 samples. Also, run a blank at the beginning and end of each batch and one spiked sample with each batch. In addition, randomly select 5-10 percent of the samples for duplicate field collection and 5-10 percent of the samples for duplicate analysis. The automated method has a reported precision of 5 µg/L. Relative standard deviation reported for the manual method ranges from 15.8 to 39.2 percent over a concentration range of 200-1,500 µg/L (APHA 1985).

DATA REPORTING REQUIREMENTS

Report results to the nearest 1 µg/L. Include the results of all QA/QC analyses with the data report.

RECOMMENDED METHODS FOR MEASURING NITRATE+NITRITE-NITROGEN

USE AND LIMITATIONS

Under aerobic conditions, ammonia can be converted to nitrite (i.e., NO_2^-) and then nitrate (i.e., NO_3^-) in the bacterial process of nitrification. Both of these inorganic nitrogen forms are highly soluble in water. In the absence of oxygen, nitrate can be converted to nitrogen gas through the bacterial process of denitrification. Because the oxidation of nitrite to nitrate is rapid, natural waters usually have very low concentrations of nitrite.

Nitrite and nitrate can be discharged directly to water from fertilizer, sewage, and manure sources. Like ammonia, nitrate is a potential source of nitrogen for plants. Therefore, increasing nitrate concentrations can stimulate eutrophication in waters in which algal growth is nitrogenlimited. This situation is more common in marine waters than in fresh waters.

In the analytical method, nitrate is first reduced chemically to nitrite and then the total nitrite is measured colorimetrically as nitrogen. Results are generally reported as nitrate+nitrite-nitrogen. The analysis is now most commonly performed by the automated cadmium reduction method, using an autoanalyzer, although the manual cadmium reduction method is also recommended. APHA (1985) Methods 418C and 418F specify procedures for the manual and automated methods, respectively. The equivalent U.S. EPA (1983) methods are 353.3 and 353.2, respectively. These methods offer the low detection limits often necessary to measure nitrate+nitrite-nitrogen in natural fresh waters of the Puget Sound region.

Nitrate-nitrogen and nitrite-nitrogen can also be determined separately. This is accomplished by splitting a sample. Nitrite-nitrogen is measured in one aliquot, and nitrate+nitrite-nitrogen is measured in the other aliquot. The nitrate-nitrogen concentration is determined by subtracting the nitrite-nitrogen concentration from the nitrate+nitrite-nitrogen concentration. A limitation of this approach is that nitrite-nitrogen concentrations may be below the analytical detection limit. If separate analysis of nitrite-nitrogen is anticipated, the sample cannot be acidified for preservation.

FIELD PROCEDURES

Sample Collection

Samples can be collected in polyethylene, polypropylene, fluoropolymer, or glass containers. A minimum sample volume of 125 mL is recommended. Samples for analysis of nitrate+nitritenitrogen can be collected in the same containers as samples intended for analysis of other variables with compatible handling and preservation (generally including ammonia-nitrogen and total phosphorus).

Sample Processing and Storage

Samples should be acidified with sulfuric acid to pH <2 and stored at 4° C in the dark. It is recommended that analysis occur within 24 h, but samples can be held up to 28 days in this condition. If samples will be analyzed separately for nitrite-nitrogen, do not acidify. Analyze as soon as possible or freeze at -20° C.

LABORATORY PROCEDURES

Automated Method

Equipment Selection and Reagent Preparation—Select equipment and prepare reagents as designated by APHA (1985) Method 418F.2 and 418F.3 or U.S. EPA (1983) Method 353.2.5 and 353.2.6. Although these procedures are based on Technicon AutoAnalyzer™ technology, other acceptable autoanalyzers (e.g., Alpkem™) exist.

Equipment Preparation—Prepare for autoanalyzer as specified by APHA (1985) Method 418F.2 and 418F.4 or U.S. EPA (1983) Method 353.2.5 and 353.2.7, or equivalent manufacturers instructions. Run a calibration curve with a blank and standards at 0.2, 0.5, and 1.0 times the expected highest concentration in a sample. The entire range of expected sample concentrations must be included in the calibration curve.

Sample Preparation—Prepare samples and standards as specified by APHA (1985) Method 418F.4 or U.S. EPA (1983) Method 353.2.7.1.

Sample Analysis—Analyze according to APHA (1985) Method 418F.4 or U.S. EPA (1983) Method 353.2.7.

Calculations— Prepare a standard curve by plotting peak heights of standards against nitrate+nitrite-nitrogen concentrations in the standards (or calculate a linear regression equation for nitrate+nitrite-nitrogen concentration as a function of peak height). Calculate sample nitrate+nitrite-nitrogen concentrations from the standard curve or regression equation.

Manual Method

Equipment Selection and Reagent Preparation—Select equipment and prepare reagents as designated by APHA (1985) Method 418C.2 and 418C.3 or U.S. EPA Method 353.3.5 and 353.3.6.

Equipment Preparation—Run calibration curve with a blank and standards at 0.2, 0.35, 0.5, 0.75, and 1.0 times the expected highest concentration in a sample. The entire range of sample concentrations must be included in the calibration curve. A reagent blank (i.e., a sample consisting of distilled water to which all reagents have been added) should be used to obtain the zero reading on the spectrophotometer prior to the analysis of standards and samples.

Sample Preparation—Prepare samples and standards as specified by APHA (1985) Method 418C.4c or U.S. EPA (1983) Method 353.3.7.8.

Sample Analysis—Analyze according to APHA (1985) Method 418C.4 or U.S. EPA (1983) Method 353.3.7.

Calculations—Prepare a standard curve by plotting absorbances of standards against nitrate+nitrite-nitrogen concentrations in the standards (or calculate a linear regression equation for nitrate+nitrite-nitrogen concentration as a function of absorbance). Calculate sample nitrate+nitrite-nitrogen concentrations from the standard curve or regression equation.

QA/QC PROCEDURES

Run control samples at 20 percent and 90 percent of the upper limit of the expected concentration range with each batch of 10 to 20 samples. Also, run a blank at the beginning and end of each batch and one spiked sample with each batch. Randomly select 5-10 percent of the samples for duplicate field collection and 5-10 percent of the samples for duplicate analysis. According to data reported by APHA (1985) for the concentration range of 0-2,100 µg N/L, the automated method has a precision of 0 to 50 µg/L and an accuracy ranging from -67 to +103 µg N/L. Precision data are only available on sewage samples for the manual method. The reported precision ranges from 4 to 10 µg N/L in the concentration range of 40 to 1,040 µg N/L (U.S. EPA 1983).

DATA REPORTING REQUIREMENTS

Report results to the nearest 1 µg/L. Include results of all QA/QC analyses with the data report.

RECOMMENDED METHODS FOR MEASURING TOTAL PHOSPHORUS

USE AND LIMITATIONS

Phosphorus can enter natural waters from sewage, detergents, fertilizers, manure, gasoline, and eroded soil. It can also be released from bottom sediments under anaerobic conditions, when iron is reduced to the ferrous form and solubilized. Phosphorus in water occurs almost solely as phosphates, including orthophosphates, condensed (poly-) phosphates, and organically bound phosphates. Phosphates occur in solution, in particles, or in the bodies of aquatic organisms.

Phosphorus is the nutrient that is most likely to limit algal growth in the fresh waters of the Puget Sound region. Therefore, phosphorus enrichment can stimulate eutrophication and result in nuisance growths of algae.

The various forms of phosphate are frequently digested to orthophosphate and expressed as total phosphorus (TP). The current trend in the Puget Sound area is to measure TP by the automated ascorbic acid reduction method using an autoanalyzer. However, the manual ascorbic acid procedure is also used. Both methods are included in the PSEP protocols. APHA (1985) Methods 424 F and G specify procedures for the manual and automated methods, respectively. APHA (1985) Method 424C (III) covers the preliminary persulfate digestion that is recommended for most analyses of natural, fresh waters. U.S. EPA (1983) Methods 365.1 and 365.2 for TP cover the automated and manual methods, respectively. These methods offer the low detection limits often necessary to measure TP in natural, fresh waters of the Puget Sound Region.

FIELD PROCEDURES

Sample Collection

Samples can be collected in polyethylene, polypropylene, fluoropolymer, or glass containers. A minimum sample volume of 50 mL is recommended. Samples for analysis of total phosphorus can be collected in the same container as samples intended for analysis of other variables with compatible handling and preservation (generally including ammonia-nitrogen and nitrate+nitritenitrogen).

Sample Processing and Storage

Samples should be acidified with sulfuric acid to pH <2 and stored at 4° C in the dark. Samples should be analyzed within 48 h, if possible, but may be held up to 28 days in this condition.

LABORATORY PROCEDURES

Automated Method

Equipment Selection and Reagent Preparation—Select equipment and prepare reagents as designated by APHA (1985) Methods 424C(III).1, 424C(III).2, 424G.2, and 424G.2.3, or U.S. EPA (1983) Methods 365.1.6 and 365.1.7. Although these procedures are based on Technicon AutoAnalyzer™ technology, other acceptable autoanalyzers (e.g., Alpkem™) exist.

Equipment Preparation—Prepare the autoanalyzer as specified by APHA (1985) Methods 424G.2 and 424G.4, or U.S. EPA (1983) Method 365.1.8.3.2, or equivalent manufacturers instructions. Run a calibration curve with a blank and standards at 0.2, 0.5, and 1.0 times the expected highest concentration in a sample. The entire range of sample concentrations must be included in the calibration curve.

Sample Preparation—Prepare samples and standards as specified by APHA (1985) Methods 424C.3 and 424G.4a or U.S. EPA (1983) Method 365.1.8.1.

Sample Analysis—Analyze according to APHA (1985) Method 424G.4 or U.S. EPA (1983) Method 365.1.8.1.

Calculations—Prepare a standard curve by plotting peak heights of standards against P concentrations in the standards (or calculate a linear regression equation for TP concentration as a function of peak height). Calculate sample TP concentrations from the standard curve or regression equation.

Manual Method

Equipment Selection and Reagent Preparation—Select equipment and prepare reagents as designated by APHA (1985) Methods 424F.2 and 424F.3 or U.S. EPA (1983) Methods 365.2.6 and 365.2.7.

Equipment Preparation—Run calibration curve with a blank and standards at 0.2, 0.35, 0.5, 0.75, and 1.0 times the expected highest concentration in a sample. The entire range of sample concentrations must be included in the calibration curve. A reagent blank (i.e., a sample consisting of distilled water to which all reagents have been added) should be used to obtain the zero reading on the spectrophotometer prior to the analysis of standards and samples.

Sample Preparation—Prepare samples and standards as specified by APHA (1985) Method 424F.4a or U.S. EPA (1983) Method 365.2.8.1.

Sample Analysis—Analyze according to APHA (1985) Method 424F.4 or U.S. EPA (1983) Method 365.2.8.1.

Calculations—Prepare a standard curve by plotting absorbances of standards against TP concentrations in the standards (or calculate a linear regression equation for TP concentration as a function of absorbance). Calculate sample TP concentrations from the standard curve or regression equation.

QA/QC PROCEDURES

Run control samples at 20 percent and 90 percent of the upper limit of the expected concentration range with each batch of 10 to 20 samples. Run blanks at the beginning and end of each batch and one spiked sample with each batch. In addition, randomly select 5-10 percent of the samples for duplicate field collection and 5-10 percent of the samples for duplicate analysis. The manual method has a reported relative precision of 4.0-9.1 percent and a relative accuracy of 4.4-10.0 percent over a concentration range of 100-7,000 µg/L (APHA 1985). The automatic method has a reported precision of 14-87 µg/L and an accuracy of -50 to 7 µg/L over a concentration range of 40-300 µg/L (U.S. EPA 1983).

DATE REPORTING REQUIREMENTS

Report results to the nearest 1 µg/L. Include the results of all QA/QC analyses with the data report.

RECOMMENDED METHODS FOR MEASURING ORTHOPHOSPHATE-PHOSPHORUS

USE AND LIMITATIONS

Orthophosphates include PO_4^{3-}, HPO_4^{2-}, $H_2PO_4^-$, and H_3PO_4. The specific orthophosphates present depend on pH. Orthophosphates are the forms of phosphorus most readily taken up by algae and aquatic plants. Therefore, it is often desirable to measure this form in addition to TP.

Samples for orthophosphate analysis should be filtered in the field to ensure that transformations to or from other phosphorus forms do not affect sample concentrations. Except for the omission of the digestion step, the same analytical methods outlined for TP can be used for orthophosphates. Orthophosphates are measured as phosphorus and are generally reported as orthophosphate-phosphorus (i.e., PO_4-P). Under test conditions, certain organic and inorganic phosphorus-containing compounds that are not orthophosphates hydrolyze and contribute to the measured orthophosphate-phosphorus concentrations. Therefore, phosphorus in filtered samples analyzed without digestion is sometimes termed soluble reactive phosphorus (SRP). As with TP, the automated method is now frequently applied, but the manual procedure is still performed. The recommended methods are APHA (1985) Methods 424F and 424G for the manual and automatic methods, respectively. Equivalent U.S. EPA (1983) procedures are Methods 365.2 and 365.1, respectively.

FIELD PROCEDURES

Sample Collection

Samples can be collected in polyethylene, polypropylene, fluoropolymer, or glass containers. A minimum sample volume of 50 mL is recommended. Samples for the analysis of orthophosphate-phosphorus can be collected in the same containers as samples intended for analysis of other variables. However, samples for orthophosphate-phosphorus analysis should be filtered in the field and then stored separately on ice in the dark.

Sample Processing and Storage

Filtered samples should be held at 4° C in the dark and analyzed within 24 h, if possible. They can be held up to 48 h in this condition. If filtration in the field is not possible, unfiltered samples can be stored on ice in the dark for a maximum of 8 h.

LABORATORY PROCEDURES

All laboratory procedures outlined in the TP protocol apply, except that digestion must be omitted. If U.S. EPA (1983) procedures are used, consult Methods 365.1.8.3 or 365.2.8.3 for the analytical procedures for orthophosphate-phosphorus.

QA/QC PROCEDURES

See recommended methods for TP.

DATA REPORTING REQUIREMENTS

Report results to the nearest 1 µg/L. Include results of all QA/QC analyses in the data report.

RECOMMENDED METHODS FOR MEASURING FECAL COLIFORM BACTERIA

USE AND LIMITATIONS

It has become the practice in water quality monitoring to measure indicator organisms rather than specific pathogens. The coliform group of bacteria is the most widely used indicator. Among that group are fecal coliform bacteria, which are found in the normal intestinal flora of warm-blooded animals, including humans.

Monitoring fecal coliform bacteria has several drawbacks. Data on fecal coliform bacteria do not provide the means to distinguish sources (PSEP 1986). Densities of fecal coliform bacteria may not accurately reflect public health risks. The recovery of these organisms from water samples may be variable and incomplete. Furthermore, their survival times in water can be shorter than those of pathogens. Nevertheless, available alternatives to monitoring fecal coliform bacteria also have drawbacks, and enumeration of fecal coliform bacteria will continue to be used to characterize water quality.

Two methods are available to measure fecal coliform densities:

- Most probable number (also called multiple tube fermentation)
- Membrane filtration.

The most probable number (MPN) method yields a statistically based estimate of bacterial density through frequency of gas production in a dilution series of fermentation culture tubes. The membrane filtration (MF) method permits direct counts of bacteria colonies that are cultured on membrane filters. The statistical reliability of the MF method has been found to be superior to that of the MPN method (APHA 1985). However, turbidity can reduce MF counts (Berger and Argaman 1983).

Recent modifications of the MF procedure have made its results compatible with MPN results (APHA 1985). Historically, the use of both methods in water quality studies in the Puget Sound region has limited comparisons of data from different sources.

Both the MF and the MPN methods are widely used, but the MF technique has more adherents among organizations active in natural freshwater monitoring in the Puget Sound region. Typically, MPN is preferred for marine waters and solid samples. MPN is also frequently used in potable water testing.

The MF procedure is recommended herein for monitoring fresh waters in the Puget Sound. This selection was made because of the widespread use of MF in freshwater monitoring and its better statistical reliability. However, it may be advisable to analyze highly turbid samples using the MPN technique. Organizations that switch from MPN to MF should conduct parallel tests to demonstrate the applicability of the method and the comparability of the data.

Full procedures for the MF technique are provided by APHA (1985) Method 909C and by U.S. EPA (1978). In U.S. EPA (1978), the delayed-incubation MF method (Part III, Section C3) is most appropriate for monitoring of natural, fresh waters.

FIELD PROCEDURES

Equipment Preparation

To prevent contamination of samples, sterile techniques must be used for all steps that involve physical contact with samples. A detailed discussion of sterile techniques is available in U.S. EPA (1978). Sterilization is typically conducted using an autoclave at 121° C for 10-30 min.

Sample Collection

Because bacteria concentrate in the surface microlayer, samples must be collected below the surface to represent the water column as a whole. Plunge the bottle 15 to 30 cm into the water upside down (if possible) to avoid the surface layer, and then turn it slightly into the current. After filling, pour out water to provide 2.5-5 cm of air space above the sample before tightly stoppering.

Sample Processing and Storage

Samples should be stored at 1-4° C in the dark. Analysis should be initiated within 6 h of collection if possible, and always within 30 h.

LABORATORY PROCEDURES

Equipment Selection and Media Preparation

Select equipment and prepare medium and buffer solutions as designated by APHA (1985) Method 909C.1 or U.S. EPA (1978) Part III, Section C3.3-5.

Equipment Preparation

Prepare for filtering as specified by APHA (1985) Method 909C.2b or U.S. EPA (1978) Part III, Section C.3.6.1-3. Sterile techniques are required for all steps that involve physical contact with samples.

Sample Preparation

Consult APHA (1985) Method 909C.2a or U.S. EPA (1978) Part III, Section C3.6.4 for guidance in selecting sample volumes for filtration. Suggested sample volumes to be filtered are as follows (U.S. EPA 1978; APHA 1985):

Lakes, reservoirs, groundwater--100 and 50 mL
Water supply intake and natural bathing waters--50, 10, and 1 mL
Farm ponds, rivers, and stormwater runoff--1, 0.1, and 0.01 mL.

Prepare the samples as specified by APHA (1985) Method 909C.2b or U.S. EPA (1978) Part III, Section C.3.6.4.

Sample Analysis

Analyze samples according to APHA (1985) Method 909C.2 or U.S. EPA (1978) Part III, Section C.3.6.

Calculations

The density of fecal coliform bacteria in a sample is calculated using the following formula:

$$\text{No. fecal coliform bacteria/100 mL} = \frac{\text{No. of Fecal Coliform colonies Counted}}{\text{Volume in mL of Sample Filtered}} \times 100$$

For best accuracy, the filter from which the data are taken should have from 20 to 60 colonies on it. If counts are not available in this range, density is determined as specified in APHA (1985) Method 909C.3.

QA/QC PROCEDURES

Randomly split 10 percent of the samples for analysis at another laboratory. In addition, randomly select 10 percent of the field samples for duplicate collection and 10 percent of the samples for duplicate analysis. Corrective actions are discussed in U.S. EPA (1978) and APHA (1985).

DATA REPORTING REQUIREMENTS

Report results to the nearest 1 colony/100 mL. Include results of all QA/QC analyses in the data report.

RECOMMENDED METHODS FOR MEASURING METALS

INTRODUCTION

This section presents recommended methods for the determination of metals at trace concentrations in the natural fresh waters of the Puget Sound region. The metals for which recommended methods are provided are listed in Table 7. These metals can be derived from both anthropogenic and natural sources. Table 7 contains typical detection limits for the most commonly applicable analytical methods, the range of concentrations for these metals in the nonindustrial rivers and lakes of the Puget Sound region, and the U.S. EPA chronic freshwater toxicity criteria. Table 7 also contains recommended detection limits for both ambient monitoring of fresh water and for assessing toxicity. To ensure data accuracy, the recommended detection limits are set at concentrations substantially lower than the expected lowest ambient concentrations and the chronic toxicity criteria.

Scientifically meaningful data for most of the metals in natural waters can only be generated using sophisticated preconcentration techniques conducted under state-of-the-art, ultra-clean handling conditions. However, U.S. EPA detection limit guidelines and chronic toxicity criteria can be met with several direct analysis techniques if sample handling is sufficiently clean. Further information on sampling devices and the minimization of contamination is available in Patterson and Settle (1975) and Bewers and Windom (1983).

Several commercial, university, and government laboratories were surveyed for currently acceptable methods prior to the development of the PSEP's recommendations for metals in fresh water. The result of this survey was that governmental and private routine testing laboratories rely almost exclusively on U.S. EPA (1983), *Methods for Chemical Analysis of Water and Wastes*. Most investigators questioned the adequacy of the methods in U.S. EPA (1983) for generating meaningful data for most metals in the non-polluted fresh waters of Puget Sound region.

The guidelines presented in the PSEP protocols document consist of simple and cost-effective methods for measuring natural levels of the metals of concern in the fresh waters of the Puget Sound region (Table 7). In general, these methods are either graphite-furnace atomic absorption (GFAA), with preconcentration (depending upon the metal), or some form of purge-and-trap preconcentration of volatile metal-derivatives followed by flame atomic absorption (flame AA) or cold vapor atomic absorption (CVAA). Inductively coupled plasma (ICP) spectroscopy is rarely capable of detecting metals at either natural or the U.S. EPA chronic toxicity concentrations. A relatively new technology, ICP-mass spectroscopy, shows promise in overcoming the problem of high ICP detection limits, but the equipment used in ICP-mass spectroscopy is expensive and not yet in general use.

If reliable background data are to be routinely generated, then greater attention will be necessary to ensure clean sampling and handling techniques. Sample contamination can occur during collection,

TABLE 7. LIMITS OF DETECTION, RECOMMENDED DETECTION LIMITS, AMBIENT DISSOLVED CONCENTRATIONS, AND U.S. EPA TOXICITY CRITERIA FOR TRACE METALS IN THE FRESH WATERS OF THE PUGET SOUND REGION (µg/L)

	Limit of Detection			Ambient Dissolved	U.S. EPA Freshwater	Recommneded Detection Limits	
Metal	ICP[a]	GFAA[b]	Other	Freshwater Concentrations[c]	Chronic Toxicity Criterion[d,e]	Ambient Monitoring[f]	Toxicity Monitoring
Aluminum	--	3	--	0.5 estim.	--	0.1-3	--
Arsenic	53	1	0.003[g]	0.3-1.2	190(As^{5+}), 48(As^{3+})	0.05-1	10
Cadmium	4	0.1	0.005[h]	0.01-0.1	1.1	0.005-0.1	0.2
Copper	6	1	0.01[h]	0.5-4.5	12	0.05-1	2
Chromium	7	1	--	0.05-0.12	210(Cr^{3+}), 11(Cr^{6+})	0.01-1	2
Iron	7	1	0.5[i]	5-120	1,000	1	200
Lead	42	1	0.01[h]	0.05-0.25	3.2	0.01-1	0.5
Manganese	2	0.5	--	0.5-100	--	0.1-0.5	--
Mercury	--	--	0.00005-0.0002[j]	0.0005-0.002	0.012	0.0002	0.002
Nickel	15	1	0.01[h]	0.05-1	160	0.01-1	30
Silver	20	0.2	0.0001[h]	0.002-0.01	0.12	0.0005-0.2	0.02
Zinc	7	0.05	--	0.5-20	110	0.1	20

[a] Reference: U.S. EPA (1984).

[b] Reference: U.S. EPA (1983).

[c] Reference: Romberg et al. (1984); Paulsen et al. (1988).

[d] Reference: U.S. EPA (1986, 1987c); the method of metal extraction is not identified in these references, and may combine data on different kinds of techniques.

[e] Criteria are hardness dependent. Values correspond to a hardness value of 100 mg/L as $CaCO_3$.

[f] Lower range is recommended based on available data; upper range is maximum recommendation based on routinely available GFAA analyses.

[g] Nonroutine method involves hydride generation, cryogenic trapping, and flame atomic absorption (Crecelius et al. 1986).

[h] Nonroutine method involves cobalt-ammonium pyrrolidine dithiocarbamate coprecipitation and GFAA (Bloom and Crecelius 1984).

[i] Method involves Fe^{2+} only, using colorometry with ferrozine (Gibbs 1976).

[j] Upper range is by routine cold vapor atomic absorption; lower range is for nonroutine method involving gold-trapping and cold vapor atomic absorption (Fitzgerald and Grill 1979; Bloom and Crecelius 1983).

handling, storage, preservation, and analysis. A particular means of sample collection or storage may be adequate for one metal but may lead to gross contamination for others. Thus, separate samples must be collected for each distinct group of metals. Alternatively, the most rigorous generally applicable sampling and storage procedures can be applied to a single sample to ensure integrity for all the metals.

USE AND LIMITATIONS

The sampling and analytical methods described in this document are appropriate for routine monitoring of metals in natural fresh waters by agencies and companies in the Puget Sound region. The methods are also appropriate for investigating and modeling biogeochemical processes. The detection limits are adequate for analysis of trace metals in drinking water, and the methods can also be used in studies of polluted, freshwater bodies. However, the methods are not intended for analysis of municipal wastes, industrial effluents, brackish waters, or other waters containing high levels of total dissolved solids that may cause unique matrix problems. Methods for dissolved and particulate phases are presented. With the exception of arsenic, chromium, and iron, speciation of trace metals cannot be determined using the recommended methods.

The methods presented herein are similar to those contained in the PSEP protocols for metals in marine water samples. Because sampling and analytical procedures and ambient concentrations of trace metals may differ between marine and freshwater environments, separate recommendations were developed for analysis of metals in fresh water.

SAMPLE CONTAINERS

The best containers for the collection and storage of water samples intended for trace metal analysis are made from fluoropolymers. Fluoropolymer containers are resistant to all known acids (even boiling $HNO_3/HClO_4$), are unbreakable, lightweight, reusable, inert, and extremely low in trace metal contamination when properly acid-cleaned (see *Cleaning Methods* section below). Freshwater samples stored in fluoropolymer containers are not contaminated by atmospheric mercury (Gill and Fitzgerald 1987; Bloom, N., 12 September 1988, personal communication). Moreover, fluoropolymer containers are also suitable for the storage of samples that will be analyzed for major constituents, nutrients, and trace organic species. Thus, although fluoropolymer containers are initially much more expensive than conventional plastic or glass bottles, their reusability, suitability for multiparameter sampling, and high degree of cleanness make them cost competitive with cheaper containers.

If fluoropolymer containers are judged too expensive, other materials may be used if special considerations are observed for each variable measured. Polyethylene and borosilicate glass are suitable for most analyses. Glass is least preferred because it is heavy, breakable, and may cause sample contamination. Polyethylene bottles cannot be used for the collection or storage of samples intended for analysis of ambient aqueous mercury concentrations. Gaseous mercury in the air readily diffuses through polyethylene and rapidly contaminates samples stored in polyethylene bottles. If fluoropolymer containers are not used for samples intended for mercury analysis, the only acceptable alternative is borosilicate glass with fluoropolymer or fluoropolymer-lined caps (Gill and Fitzgerald 1987).

Flint glass, soft glass, and lead glass should never be used for trace metal samples. These materials may be high in acid-leachable lead, iron, zinc, and manganese from the minerals in the glass. Under no circumstances should aluminum, cardboard, cork, or rubber caps or liners be used with trace metal samples. Polyvinyl chloride (PVC) containers should be avoided because this material is often high in entrained contaminants. Nylon containers should be avoided because nylon is readily degraded by acid.

SAMPLING EQUIPMENT

Sampling equipment should be made from the same materials listed above for containers. The equipment should not contain metal or rubber components. However, silicone rubber is acceptable. O-rings, springs, and other parts should be fabricated from silicone rubber, fluoropolymers, or titanium. As a last resort, exterior components of a sampling device may be constructed from stainless steel and coated with plastic material. If sampling is to be conducted by hand-dipping, the hands of the person collecting the samples should be covered with shoulder-length, plastic, clean-room gloves. If a dipping-ladle is used, then the same restrictions that apply to the materials for containers also apply to the materials for dipping-ladles. Remote sample collection bottles and pumping systems must be cleaned to the same specifications as the storage containers. The hydro-line and weight used to lower a sample collection device into the water must be made from or coated with plastic. Kevlar™ is the ideal material for hydro-line due to its great strength and ability to stay clean. See Bewers and Windom (1983) for an intercomparison of sampling devices for trace metals.

Sampling bottles commonly employ closing mechanisms and seals that are incompatible with trace-metal sampling, although they are adequate for major element and nutrient monitoring. Any sampler containing metal or rubber components on the interior should never be used. Exterior parts may be made of silicone rubber, 316 stainless steel, or titanium. The best sampling bottle for trace-metal analysis is the fluoropolymer-coated Go-Flo™ bottle (General Oceanics, Miami, FL).

CLEANING METHODS

Sampling devices, containers, pipette tips, and GFAA sample cups should be cleaned as rigorously as their material will allow, and then appropriate measures should be taken to maintain cleanness. Fluoropolymer containers should be cleaned by soaking in hot (60-95° C) concentrated HNO_3 for 24-48 h, followed by 24 h in hot, dilute, high purity HNO_3 (i.e., ultrapure or equivalent) that is low in trace metals. Polyethylene and glass containers should be soaked in 6N HNO_3 for 1 wk, and then rinsed thoroughly with high-purity deionized water (i.e., water that has been shown to be low in trace metals). After rinsing, the containers may be stored filled with dilute high-purity acid, shaken out and stored wet, or dried in a clean air station prior to closure. All clean containers should be stored in a dust-free environment or clean, dry, plastic bag. For more information on cleaning, see Bloom and Crecelius (1983). An alternative to cleaning containers used previously, is to purchase pre-cleaned containers for each sampling excursion.

Sampling devices containing silicone, PVC, or less robust plastic parts should be soaked for 24 h in 10 percent HNO_3 or HCl, and then should be thoroughly rinsed with deionized water. No hidden nylon, metal, or rubber components can be present on a device that is soaked in acid. Stainless steel can be rinsed briefly in 10 percent HNO_3, and then it should be rinsed thoroughly with deionized water prior to drying in a Class 100 clean air station.

FIELD PROCEDURES

Sample Size

The sample volume needed for trace metals analysis depends upon the analytical techniques to be used. If only direct injection atomic absorption or ICP are to be used, then 100-mL samples will be sufficient. A 2-L sample is adequate for the accurate determination of all U.S. EPA priority pollutant metals in a freshwater sample. Several liters of water may be needed to obtain enough particulate matter for analysis. Larger volumes are needed when preconcentration techniques are used prior to analysis, especially since a different aliquot of water may be needed for each of several methods.

Sample Collection

Freshwater samples may be collected by a variety of means, depending upon the scientific and analytical rigor required. The sampling device must be constructed of a material compatible with the metals being analyzed, and it must be rigorously cleaned as discussed above. Acid-cleaned and rinsed sampling devices should be stored in a clean polyethylene bag for field use. To estimate contamination from the sampling device, high-purity water can be stored in the sampling device for an appropriate period of time and then be analyzed.

Surface samples are collected by dipping with a sampling ladle or directly with a sample bottle. To minimize contamination, the person collecting the sample should wear clean-room grade, polyethylene gloves. Dipping is performed while facing into the direction of water flow (i.e., while standing in the stream, off the bow of a moving boat, or on the upstream side of a bridge). The sampler should be thoroughly rinsed with the water being sampled. The rinse water should be discharged downstream prior to sampling. Avoid sampling in obvious patches of surface scum. The sampler must be completely immersed to prevent inadvertent collection of material from the surface microlayer.

If samples are to be collected at depth in a water body, then a specialized sampler or a pumping system must be used. Samplers generally consist of a cylindrical tube with a stopper at each end and a remotely activated closing device. Closure is activated by dropping a weighted messenger down the line or by sending an electrical signal from shipboard. The Kemmerer, Van Dorn, Niskin, Go-Flo™, and Nansen samplers are commonly used to obtain water samples for metals analysis. Each device samples a discrete volume of water (from 2 to 30 L). Sampling depth is controlled by the length and angle of hydro-line released from a winch. Multiple water samplers may be fixed to the hydro-line or on a rosette frame, so that several depths or replicates can be sampled on a single hydrocast.

Prior to deployment, the stoppers of the sampler are cocked open. It is critical at this point to avoid contamination of the sampler interior and stoppers. The sampling team should wear clean-room gloves, and avoid touching the interior of the bottle and setting it on unclean surfaces. Because ship decks and highway bridges are often very dirty, continuous vigilance is necessary to avoid contaminating the sampler.

After the sampler is cocked, it is lowered to the desired depth. The sampler is lowered with both ends open, so that ambient water flows through the device as it passes through the water column. When the sampler is at the desired depth, it should be allowed to equilibrate with ambient conditions for 2-3 min before the closing mechanism is activated. The sampler then is raised to the surface. Once the sampler is brought out of the water, the stoppers or valves should be checked immediately for complete closure. If the sampler has not properly sealed, the sample cannot be used.

Care should be exercised to avoid contamination of the sampler as it passes through the surface microlayer. The sampler should not be deployed through surface slicks. Some samplers are designed to avoid contamination from the microlayer because they can be deployed with the stoppers closed (e.g., Go-Flo™ Bottle). On such samplers, a pressure sensor triggers the opening of the stoppers at a depth of about 10 m.

It is recommended that at least two samplers be used simultaneously at each depth, both to provide a backup if one device does not close properly, and to provide a larger sample volume from precisely the same location in the water body. Multiple casts made using one water bottle to the same depth will not be true replicates because of between-sample drift and currents.

For sampling shallow water bodies (<30-m depth), contamination problems are generally less severe when samples are collected by pumping rather than by the samplers described above. Typically, a 1/4-in outside diameter, flexible, acid-cleaned, fluoropolymer tube is lowered to the desired depth using a fluoropolymer weight. Water is pumped through the tube and into the sampling container by vacuum. An acid-cleaned, silicone tubing, peristaltic pump may also be used. Pumping methods have the advantage that the sample can be filtered as it is collected, using an in-line fluoropolymer filter holder with an acid-cleaned, polycarbonate, membrane filter or acid-cleaned, fluoropolymer filter.

Sample Processing and Storage

Sample processing may involve filtration, preservation, and transportation. Samples to be analyzed for total metals (dissolved plus particulate) are preserved in the field. Samples to be analyzed for dissolved or particulate metals must undergo separation of the phases (typically by filtration) prior to preservation.

Filtration—Several methods are available for separating the dissolved and particulate phases of water samples. These methods include filtration, settling, batch centrifugation, and continuous flow centrifugation. However, continuous flow centrifugation is inappropriate for metals analysis because of the metallic components used in the construction of the equipment. All devices used to separate particulate from dissolved materials may contaminate the sample, especially the dissolved phase. Careful attention must be given to minimize contamination. Quantification of blanks is necessary if accurate results are to be obtained.

Filtration is the preferred method of particle separation for aqueous samples. Compared with other methods, filtration gives a more precisely defined fractionation, can handle larger samples, and requires relatively inexpensive equipment. The particulate fraction is also much more easily removed from filters than from centrifuge bottles. Centrifugation may be preferable for samples containing large amounts of suspended material due to the relatively small quantity of suspended matter that may be collected on a filter before clogging.

Currently, the most common definition of the dissolved phase is the material that passes through a 0.45-µm filter. However, the water column contains a substantial amount of smaller particulate matter, which ranges in size down to colloidal materials (i.e., size range of 0.001-1 µm). Thus, if a filter with a smaller pore size is employed, the proportion of metals designated as dissolved will decrease, while the proportion designated as particulate will increase. The concentrations of metals that are present in relatively high concentrations in colloidal materials, such as iron, aluminum, and manganese are most likely to vary with filter pore size. Other metals, such as arsenic, cadmium, zinc are primarily in the dissolved phase.

Although the most common definition of the dissolved phase is the material that passes through a 0.45-µm pore size filter, several types of filters often used in the Puget Sound region have a nominal pore size of 0.4 µm. In practice, there is probably little difference in the material retained by filters with 0.45- and 0.4-µm pore sizes.

Samples may be filtered using vacuum or positive pressure. Acid-cleaned polycarbonate membrane filters are used. The filters are cleaned in 6N HNO_3 for at least one week, and then are stored dry in a clean container, dry in a preassembled filter holder, or in a jar containing deionized water. The advantages of polycarbonate filters are their uniform pore size, durability in handling, and highly reproducible dry weight. The major disadvantage of these filters is that they clog rapidly when filtering organic-rich, fresh water. As little as 100 mL of eutrophic lake water may clog a 47-mm diameter filter. Larger samples may be processed using larger filters (e.g., 142-mm diameter).

More porous filter media are used to filter larger water volumes. For a given pore size, the most porous filters are made from glass or quartz fibers. Workshop attendees noted that these filters suffer from poor pore-size definition, sample contamination for some metals, and fragility. Glass fiber and cellulose filters cannot be weighed accurately enough after use to quantify the small mass of suspended matter typically collected. A good compromise filter is made from cellulose nitrate or acetate. These filters may be mildly acid-cleaned using 10 percent HNO_3, and they can be used to filter up to 10 times the sample volume that can be filtered using an equivalent polycarbonate filter. If both large sample volumes and a measure of suspended load are needed on the same sample, then one aliquot should be filtered through a polycarbonate membrane for weighing, and the rest of the sample should be filtered through a more porous filter.

When a sample is filtered, the first 100-1,000 mL of filtrate should be discarded to allow a final rinsing of the filter before collection of the dissolved sample. If the filtration is conducted in a laboratory, the filter may be pre-rinsed with dilute HNO_3 followed by copious amounts of deionized water prior to actual sample filtration. The sample should be periodically agitated to ensure a homogenous distribution of the suspended matter as filtration proceeds.

Metals may be measured as total metals, dissolved metals, and particulate metals. Data for any two of these fractions will allow calculation of the other fraction. However, analysis of all three fractions provides a mass-balance check of the overall filtration integrity and a check that the appropriate blanks were taken into account. Several filter and filtrate blanks should always be analyzed because the variability introduced by filtration contamination can be substantial. Filtration blanks should be taken in the field. Filtration blanks are high purity deionized water that is filtered exactly as are samples. For accurate blank determination, water that was poured into the filter and water that passed through the filter should both be preserved for later analysis.

After filtration is completed, filters may be stored either within the pre-assembled filter holder for laboratory disassembly, or removed in the field using fluoropolymer-coated tongs and placed into storage containers. Filters may be placed into small, acid-cleaned vials for direct acid digestion, or they may be stored in a flat position in appropriately sized, polystyrene petri dishes for later drying at 80° C and accurate weighing before analysis. If the weight of the particulates is to be determined, the clean filters must be initially dried in individually numbered petri dishes, and accurately weighed before sample collection. Because polycarbonate filters are prone to static charge buildup after drying, a positive-ion generator must be used to neutralize the charge on the filter surfaces prior to weighing.

With proper cleaning and assessment of blanks, disposable filter units are often most convenient when a small number of samples is to be filtered. The particulate phase on these units is not recoverable, however, and the cost-per-sample is high.

Preservation—Filtered and unfiltered water samples are typically preserved in the field by the addition of acid. Acid stops biological activity and minimizes adsorption of ionic substances to the bottle walls. Because acid dissolves metals in the particulate phase, samples that will be filtered should not be acidified before filtration. The dissolved fraction is acidified after filtration. For most freshwater samples to be analyzed for trace metals, acidification to a pH <2 by adding 1-mL ultrapure or equivalent HCl or HNO_3 per liter of sample is sufficient to preserve the sample for an extended period without affecting blanks. It is preferred in many laboratories to acidify to 1 percent or even 5 percent on a volume:volume basis. This level of acidification is unwarranted for preservation purposes. Also, such acidification makes virtually impossible most preconcentration techniques because they are strongly dependent upon pH.

If samples cannot be filtered in the field, they should be stored (without acidification) on ice in the dark. Filtration and preservation should be completed within 24 h of collection.

Samples to be analyzed for mercury are best preserved by the addition of 5 mL of bromine monochloride/HCl solution per liter of sample (Bloom and Crecelius 1983). If this reagent is unavailable, then 10 mL HCl (not HNO_3) per liter of sample should be used as a preservative, because chloride complexation helps prevent Hg^{++} from reducing to Hg in solution.

Acids used for preservation should be ultrapure or equivalent, suitable for trace metal analysis, and sufficiently low in trace metal concentrations for the needs of the study being conducted. Care must be taken to avoid cross-contamination of the preserving acid in the field. Do not insert dirty pipette tips into the preserving acid and, when possible, measure the acid aliquot by pouring into a scrupulously clean, graduated fluoropolymer vial. Pipette tips must be acid cleaned, and colored tips should not be used because some of the coloring agents contain metals.

Samples may also be preserved for later analysis by rapid freezing in liquid nitrogen (Crecelius et al. 1986). This method should be used if the oxidative state of metals is to be determined or if organic forms of metals (e.g., methyl mercury) are to be analyzed. Acids generally alter the metal speciation of a sample. If water samples are to be returned to the laboratory on the collection date, they may be preserved in the laboratory. Otherwise, samples should be preserved in the field.

Once the sample is collected and preserved, the sample bottle should be capped and stored in a dust-free environment, including at least a double polyethylene bag to prevent meltwater from contaminating the sample, for shipment back to the laboratory. Samples should not be packed in vermiculite or other potentially contaminating particulate matter. Filters may be taken to the laboratory for drying and weighing. Filters can be preserved in the field by freezing or the addition of HNO_3 to the filter, which is held in an acid-cleaned fluoropolymer or glass vial.

Holding Times—Samples should be analyzed as soon as possible. Maximum holding times for preserved samples intended for metals analysis are 24 h for Cr^{+6}, 28 days for mercury, and 6 months for other metals.

LABORATORY PROCEDURES

This section presents information on sample preparation, analytical methods, and specific element quantification for metals in the fresh waters of the Puget Sound region.

Sample Preparation

U.S. EPA protocols recommend a hot oxidizing digestion for both unfiltered water samples and filtered samples that form a precipitate upon acidification. This step causes release of all particle-bound metals prior to analysis. However, the recommended procedures are prone to gross contamination of ambient freshwater samples. Contamination may come from evaporation of the sample in an open beaker, filtration of the acidic/oxidizing solution, and the additions of large amounts of reagents.

The procedures for hot oxidizing digestions (see Exhibit D of U.S. EPA 1987a) can yield much better results if the following precautions are observed:

- Conduct all operations, especially the open-beaker digestion, in a Class 100 clean-air station. Class 100 criteria are concerned with maintaining low particle density in the air.
- Pre-analyze all reagents to confirm purity.
- Do not filter acid-digested samples. Let acid-digested samples settle and analyze the supernatant liquid.
- Run at least three complete procedural blanks for every 20 samples prepared together or for batches of fewer than 20 samples. These blanks provide a statistically meaningful measure of the variability introduced by contamination that may have occurred during sample handling. Alternatively, pooling duplicate blank data from a series of sample batches can be used to assess the variability of blank contamination at a laboratory.

Because of the great risk of contamination, many investigators recommend that no sample pretreatment (other than acidification) be utilized for water samples that will be analyzed by high-temperature spectrographic techniques (e.g., AA, ICP, flame emission spectroscopy). The rationale for this recommendation is that virtually all trace metals of biogeochemical importance are leached from particles at pH 2. The small fraction of nonacid-leachable metals that remains unaccounted for after acidification is much less important than the amount of contaminants that may be introduced into samples during use of the currently accepted digestion procedures.

Preconcentration of Metals from Water Samples—A preconcentration step is used to obtain accurate values for metals that are found at concentrations less than the detection limits of direct analysis techniques. Several preconcentration techniques are recommended below. An advantage of preconcentration is that it also generally eliminates matrix effects in later analysis because the metals are in a simple solution. Unfortunately, no preconcentration technique is adequate for all metals, so a variety of individual methods must often be employed. If GFAA analysis is used for the analysis of fresh water, no preconcentration step is needed for iron, manganese, or zinc.

When water samples are to be preconcentrated, some form of sample digestion is often warranted. This step may be necessary to break down organometallic complexes from which metals might otherwise be incompletely extracted. This step is particularly important for mercury analysis using $SnCl_2$ reduction and CVAA. In fresh waters, a large fraction of mercury is strongly bound to complex dissolved organic materials and is not completely converted to gaseous mercury by the $SnCl_2$ reaction. Thus, water samples intended for mercury analysis must be pre-oxidized, usually with a free halogen or hydrogen peroxide. Simple and efficient oxidation techniques for breaking mercury-carbon bonds include bromine monochloride/hydrochloric acid (Bloom and Crecelius 1983) and potassium persulfate (U.S. EPA 1983).

Chelate-coprecipitation (Appendix A), solvent extraction techniques (Danielsson et al. 1978), and volatilization by hydride generation (Appendix B) can provide good recoveries when used for preconcentrating metals from undigested water samples. When ion-exchange preconcentration is used, natural chelating agents can prevent trace metals from adsorbing to the chelating resin unless the sample is first digested. Samples that are digested by wet oxidation techniques must be pre-reduced (e.g., by the addition of hydroxylamine hydrochloride) before organic chelation reactions are attempted. Otherwise, the chelating agents can be destroyed by residual oxidizing species in the digested sample.

Several chelation techniques are available that allow the simultaneous preconcentration of silver, cadmium, copper, lead, and nickel by complexation with ammonium pyrrolidine dithiocarbamate (APDC). These methods may rely upon complexation followed by solvent extraction or coprecipitation with a nonanalyte carrier metal (e.g., cobalt). These steps are followed by filtration and dissolution in acid (Boyle and Edmond 1975; Bloom and Crecelius 1984). The coprecipitation method is advantageous because it is suitable for "mass-production" techniques, and it renders the highly concentrated (approximately 20-100X) extract in a simple HNO_3 matrix.

Most of the transition metals can be simultaneously preconcentrated using cationic chelating resins such as Chelex-100 (Kingston et al. 1978; Bruland et al. 1979; Paulson 1986) followed by back-elution into dilute acid. Ion exchange methods are time consuming and provide low concentration factors because of the relatively large elution volume of acid needed.

All chelation/ion exchange methods of preconcentration are extremely pH dependent. Samples should be reproducibly acidified with the minimum acid necessary to keep the metals in solution, so that a minimum quantity of buffer is required to bring the sample to the appropriate pH for extraction. The cobalt-APDC coprecipitation, $SnCl_2$ mercury reduction, and hydride generation techniques all work very well at a pH of 1.8. This pH can be achieved when samples are acidified with 1.0 mL HCl (12.2 N) per liter of water.

Other techniques may require the addition of buffers to bring the pH to more favorable levels (i.e., 4-6) prior to extraction. To avoid contamination, a pH probe should never be inserted into a sample that will be analyzed. A separate aliquot of the sample must be used for pH determination. The calculated amount of buffer needed is then added to the clean subsample to be extracted. Buffers are potential sources of contaminants and must be certified low in metals concentrations prior to use. Trace metals can be removed from buffers by APDC/solvent extraction or ion-exchange purification.

Arsenic, antimony, chromium, beryllium, and selenium may be preconcentrated by co-precipitation with $Fe(OH)_3$ at pH 8.6. High purity Fe^{+3} solution is added to bring the sample to approximately 10^{-3} M Fe^{+3}. Ammonium acetate/ammonium hydroxide buffer is then added to bring the final pH to approximately 9.0. After occasional swirling for several hours, the sample is filtered to remove the $Fe(OH)_3$ with the adsorbed metals. The precipitate is then redissolved in 1.0 mL concentrated HNO_3, and diluted to approximately 5.0 mL with deionized water for analysis.

Another class of preconcentration methods involves volatilization of the metal and sweeping from solution by bubbling. Mercury may be directly volatilized by the addition of acidic $SnCl_2$ or aqueous sodium borohydride ($NaBH_4$), while arsenic, selenium, and antimony may be volatilized as the hydrides by reaction with $NaBH_4$. All these reactions occur best at low pH (approximately 1-2). The volatile gases may then be swept directly into a detector such as CVAA for mercury analysis or an air/hydrogen flame AA system for analysis of the other metals.

Much greater analytical sensitivity can be obtained by preconcentrating the volatilized species on a trap prior to analysis. This technique can provide concentration factors as high as 1,000X. Volatilized mercury is precollected by amalgamation onto gold or gold-coated sand (Fitzgerald and Gill 1979; Bloom and Crecelius 1983). The mercury is then released into the detector by thermal desorption. The metalloid hydrides may be collected into a balloon or syringe, or onto a cryogenic trapping column held in liquid nitrogen (Andraea et al. 1981; Crecelius et al. 1986). In the balloon or syringe methods, the balloon or syringe is squeezed to rapidly release the entire sample into the flame, while in the cryogenic trapping column method, thermal desorption is used. For arsenic, antimony, and selenium, the cryogenic trapping technique also allows determination of the oxidative state and analysis of organometallic forms.

Particulate Samples—Filters for particulate matter may be dried to constant weight at 80° C in a clean-air oven to determine the total suspended mass collected. This procedure requires that the samples be collected on polycarbonate filters that had been preweighed in the same manner. After weighing, these samples can be analyzed for all metals except mercury. The hot HF/aqua regia digestion used for sediments is recommended [see section on sediments in the PSEP protocols document on metals (PSEP 1989)]. Samples collected on cellulose acetate or glass fiber filters, or those that will be analyzed for mercury, should not be dried. These samples should be wet-digested in small fluoropolymer or glass vials by one of the approved methods for sediments and then diluted to approximately 5 mL prior to analysis.

In several laboratories in the Puget Sound region, dry intact filters are analyzed directly for many metals by x-ray fluorescence (XRF) spectroscopy (Feely et al. 1986). However, the detection limits for several metals using XRF (e.g., silver, mercury, and cadmium) are too high to be useful. Thus, when XRF is used, analysis of these metals requires that an additional filter be collected and digested for GFAA analysis.

Analytical Methods

In general, the only commonly available technique capable of accurately quantifying the U.S. EPA priority pollutant metals in ambient fresh waters is GFAA. The methods most commonly adhered to by routine analytical laboratories for GFAA analysis are those published by U.S. EPA (1983). Unfortunately, these methods are out of date compared with current instrumentation and techniques. In general, the following additional guidelines for GFAA analysis should be considered until updated standard protocols are published.

- GFAA sample injection should be performed in all cases using an auto-sampler. This single step can reduce intersample variability by at least 1 order of magnitude compared with hand injection techniques.

- Matrix modification (i.e., a pre-analysis step that changes the chemical nature of the sample matrix) of some type is almost always warranted to reduce intersample variability, reduce matrix interference, and increase sensitivity (Manning and Slavin 1983). For the following matrix modifiers, 5 µL of the matrix modifier can be added to a 20-µL aliquot of sample (other matrix modifiers also have been helpful in some cases):

  - 2 percent $NH_4H_2PO_4$ is used for the low-boiling metals (cadmium, copper, lead, zinc, and silver)

  - 0.5 percent $Mg(NO_3)_2$ is used for the refractory, oxide-forming metals (aluminum, beryllium, chromium, and manganese)

  - 0.5 percent $Ni(NO_3)_2$ is used for the metalloids (arsenic, antimony, and selenium).

- Electrodeless discharge lamps should be used in place of hollow cathode lamps where possible. Advantages of electrodeless discharge lamps include their greater brightness and baseline stability.

- Background correction should be routinely used, if possible. Advanced background correction (e.g., Zeeman effect, Smith-Heifje) is possible at all wavelengths and for very high background levels. The Zeeman effect and Smith-Heifje background correctors are more effective than the D_2 arc background corrector.

- Preconcentration of the metals of interest should be conducted by extraction, coprecipitation, or volatization when warranted. Preconcentration not only increases sensitivity, but also vastly reduces the variability introduced by matrix interference.

- Pyrolytic L'vov platform atomization with "maximum-power" temperature control (Slavin et al. 1984) should be used to obtain more accurate and reproducible results. The instrument parameters given in U.S. EPA (1983) are primitive and inflexible compared with the fine-tuning available using this technique.

After GFAA, the next best category of analytical techniques involves preconcentration by volatilization of the element followed by flame AA or CVAA. Such techniques are applicable to arsenic, antimony, selenium, and mercury and provide increases in sensitivity of up to 1,000X. These methods typically require considerably more time and operator skill than direct injection techniques, however.

Other analytical techniques may also produce acceptable data if proper QA/QC procedures are followed. ICP and XRF are two other commonly used methods in analytical laboratories. These techniques, while excellent for metal-rich samples such as tissues and sediments, are not generally useful for ambient water monitoring. ICP and XRF are often used in the analysis of particulate-phase metals. ICP can be used directly for iron and manganese analyses in many water samples, and can be used for several other metals following 50-100X preconcentration. If ICP analysis is used, potential spectral interferences from other matrix constituents should be assessed before interpreting the data for low-level multi-element analysis (U.S. EPA 1987a). Colorimetric techniques [i.e., ferrozine determination of Fe^{+2}] or gas chromatography methods may also occasionally be used for specific metal/matrix combinations.

Using an analytical technique for samples with metals concentrations near the detection limit should generally be avoided because values obtained near the detection limit may be erroneous. This phenomenon may lead to agency or community acceptance of data on background metals concentrations that indicate higher or lower concentrations than are actually present in the environment. It is recommended that analyses be conducted at a minimum of at least 3 times the detection limit (based on procedural blanks for an analyte). This recommendation should be followed even if it is necessary to switch to a different technique or preconcentration procedure for a particular group of samples.

Specific Element Quantification

Information is presented below concerning analytical methods for determination of specific metals in freshwater samples. The metals discussed are aluminum, arsenic, cadmium, chromium, copper, iron, lead, manganese, mercury, nickel, silver, and zinc.

Aluminum—Aluminum cannot be determined in filtered freshwater samples by direct aspiration ICP analysis because the detection limit of 45 µg/L is about 100 times higher than ambient concentrations. ICP analysis at a wavelength of 308.215 mn is suitable for the analysis of HF/aqua regia-digested, suspended matter samples. Aluminum can be determined at ambient levels using direct injection GFAA if care is taken to avoid contamination. Using U.S. EPA Method 202.2 (Table 8), a detection limit of about 3 µg/L is attainable. This detection limit may be lowered to about 0.1 µg/L if a newer instrument employing maximum power atomization is used in conjunction with pyrolytic graphite tubes. A matrix modifier of 0.5 percent $Mg(NO_3)_2$ is also useful in reducing intersample variability. Under the above conditions, the char temperature may be raised to 1,500° C and the maximum-power atomization temperature reduced to 2,500° C (Table 8).

The concentration of aluminum is very high in suspended matter (approximately 2-8 percent), so that detection is not difficult. The HF/aqua regia digestate of suspended matter usually must be diluted for on-scale reading by GFAA. Care must be taken to avoid contamination when large dilutions of a sample are made. The contaminant concentration is multiplied by the dilution factor when the sample concentration is calculated

Arsenic—Arsenic cannot be determined in natural fresh waters by ICP direct aspiration analysis. The detection limit of this method is greater than ambient concentrations and the U.S. EPA chronic toxicity criterion for As^{+3}. Available atomic absorption methods include U.S. EPA Method 206.2, which is a GFAA method with a detection limit of 1 µg/L, and U.S. EPA Method 206.3, which is a hydride generation/flame AA technique with a detection limit of about 2 µg/L. These methods both use the 193.7 nm arsenic line. Much better baseline stability and detection limits are available when an electrodeless discharge lamp is used instead of a hollow cathode lamp.

For the GFAA method, use of 0.5 percent $Ni(NO_3)_2$ as a matrix modifier allows a char temperature of 1,100-1,200° C. Atomization temperature may be reduced to 2,500° C if a maximum power atomization step is available. The method of standard additions and advanced background correction (e.g., Zeeman effect) should be used if possible. Aluminum can strongly interfere with arsenic measurements when analyses are conducted by GFAA. In particulate digestates, the following parameters have been used successfully. The 197.2 nm resonance line is used, and 100 mg/L aqueous palladium solution is used as a matrix modifier (Xiao-quan et al. 1984). The L'vov platform is used with a char temperature of 1,400° C and an atomization temperature of 2,600° C. The detection limit for this method is about 0.2 µg/L.

TABLE 8. GUIDELINES FOR THE MAJOR PARAMETERS USED FOR GFAA DETERMINATION OF TRACE METALS IN FRESHWATER SAMPLES[a,b]

Metal	U.S. EPA Method	Char Temp[c] (°C)	Atomization Temp[d] (°C)	Lamp[e]	Wavelength (nm)	Suggested Matrix Modifiers[f]
Aluminum	202.2	1300	2700	HCL	309.3	0.5% $MgNO_3$
Arsenic	206.2	1100	2700	EDL	193.7	0.5% $NiNO_3$
Cadmium	213.2	500	1900	EDL	228.8	2% $NH_4H_2PO_4$
Chromium	218.2	1000	2700	HCL	357.9	0.5% $MgNO_3$
Copper	220.2	900	2700	HCL	324.7	2% $NH_4H_2PO_4$
Iron	236.2	1000	2700	HCL	248.3	--
Lead	239.2	500	2700	EDL	283.3	2% $NH_4H_2PO_4$
Manganese	243.2	1000	2700	HCL	279.5	0.5% $MgNO_3$
Mercury	245.1	--------Cold Vapor AA--------			254.1	--
Nickel	249.2	900	2700	HCL	232.0	--
Silver	272.2	400	2700	HCL	328.1	2% $NH_4H_2PO_4$
Zinc	289.2	400	2500	EDL	213.9	2% $NH_4H_2PO_4$

[a] Reference: U.S. EPA (1983).

[b] Argon purge gas and advanced background correction are assumed.

[c] Assumes use of Perkin-Elmer HGA-2100 with a simple HNO_3 matrix. Char temperature may differ if other matrix modifiers are used.

[d] Assumes use of Perkin-Elmer HGA-2100 with a simple HNO_3 matrix. Atomization temperature may differ if other instruments are used.

[e] HCL - hollow cathode lamp; EDL - electrodeless discharge lamp.

[f] Reference: Manning and Slavin (1983).

Hydride generation is an excellent technique for measuring total inorganic arsenic (see Appendix B). This method provides clean peaks with high sensitivity. The detection limit using U.S. EPA Method 206.3, which sweeps the AsH_3 directly into the flame as it is generated, is about 2 µg/L. If a cryogenic trap is employed to collect all of the generated AsH_3 prior to atomization, a detection limit of about 0.003 µg/L can be obtained (Crecelius et al. 1986; Andraea et al. 1981). In this latter technique, the sample (approximately 20 mL) is adjusted to pH 1-2. In a bubbler vessel, 3 mL of 1 percent $NaBH_4$ solution is slowly injected through a septum under the water surface. As the AsH_3 is generated, it is purged from the system with helium and collected by condensation in a U-tube trap. The U-tube trap is packed with 15 percent OV-3 on chromosorb WAW-DMSC and held in liquid nitrogen. Upon electrical warming, the arsenic hydrides (including organoarsenic species, if present) are eluted from the trap according to boiling point, and are detected by air/H_2 flame AA.

As^{+3} and As^{+5} can be individually determined using hydride generation by varying the parameters of reaction. In U.S. EPA Method 206.3, total arsenic is determined using the addition of $SnCl_2$, and As^{+3} is determined on a separate aliquot without $SnCl_2$ addition. Using the cryogenic trapping technique, total arsenic is determined at pH 1-2, while As^{+3} is determined on a separate aliquot at pH 4. Arsenic species can also be quantified by ion chromatography, although the detection limit is about 10 µg/L.

Arsenic ion acid-digested filter samples can be measured by GFAA at about 0.1 µg/g [assuming a 10 mg (dry weight) sample of particulate matter is digested and the digestate diluted to a final volume of 5.0 mL]. Using hydride generation/cryogenic trapping, a detection limit of at least 0.03 µg/g is possible. However, this sensitivity may not be necessary because typical concentrations of arsenic in suspended material are several µg/g. XRF spectroscopy of intact dry filter media has also been successfully applied to the determination of total arsenic in suspended matter.

Cadmium—Direct aspiration ICP analysis of cadmium, which has a detection limit of about 4 µg/L, is inadequate for the quantification of cadmium at natural concentrations or at the U.S. EPA freshwater chronic toxicity criterion. GFAA using the guidelines of U.S. EPA Method 213.2 (Table 8) has a detection limit of about 0.1 µg/L, which is sufficient to detect concentrations at the chronic toxicity criterion for this element in fresh water. Many researchers report improved reproducibility and fewer matrix interferences when using L'vov platform atomization instead of the commonly used technique. The L'vov platform is used with a maximum power atomization temperature of about 2,000° C and a char temperature of about 800° C. The 2 percent $NH_4H_2PO_4$ matrix modifier must also be used.

For accurate quantification of ambient cadmium concentrations in fresh water, the sample should first be preconcentrated by a factor of 20-50X. The cobalt-APDC coprecipitation method (Boyle and Edmond 1975; Bloom and Crecelius 1984) is recommended for this purpose (Appendix A). It is rapid, efficient, and provides accurate results. The solution produced from use of this method already contains $NH_4H_2PO_4$, which further saves analytical time.

Cadmium in acid-digested filter samples can be determined at a concentration of about 0.05 µg/g [assuming a typical 10 mg (dry weight) particulate sample is digested and the digestate is diluted to a final volume of 5.0 mL]. Phosphate matrix modification, the L'vov platform, and advanced background correction (i.e., Zeeman effect or Smith-Heifje) are necessary for good analytical reproducibility in this matrix.

Chromium—Direct aspiration ICP analysis is not sensitive enough to quantify chromium in natural freshwater samples, although it is sensitive enough to meet the U.S. EPA freshwater chronic toxicity criterion of 11 µg/L for Cr^{+6}. (Caution, Cr^{+6} samples have a holding time of only 24 hours.) Using GFAA as outlined in U.S. EPA Method 218.2 (Table 8), chromium may be measured at a concentration of about 1 µg/L. Accurate quantification of ambient concentrations requires detection limits that are approximately one order of magnitude lower. The direct injection detection limit can be improved to approximately 0.2 µg/L through the use of a 0.5 percent $Mg(NO_3)_2$ matrix modifier and maximum power atomization. If $Mg(NO_3)_2$ with a sufficiently low blank concentration is unavailable, clean filtered seawater serves as an excellent alternative modifier. Using these modifications to the U.S. EPA Method 218.2, a char temperature of 1,300° C and maximum-power atomization temperature of 2,500° C are recommended.

Cr^{+6} may be determined on separate sample aliquots by selective extraction of the Cr^{+6}. Cr^{+3} is then determined by subtraction from the total chromium value. U.S. EPA Method 218.4 contains an extraction technique for Cr^{+6} using solvent extraction of the APDC complex at pH 2.4. The solvent phase should be back-extracted into acid prior to analysis by GFAA. According to the procedure outlined in U.S. EPA Method 7195, Cr^{+6} may also be selectively extracted as $PbCrO_4$ using coprecipitation with $PbSO_4$. The coprecipitate is redissolved in HNO_3 prior to analysis by GFAA. The determination of Cr^{+6} in water by colorimetry of the red chromium-diphenylcarbazide complex has poor detection limits and is subject to interferences from other metals. Analysis of chromium speciation may also be possible by ion chromatography.

Chromium in HF/aqua regia-digested, particulate matter may be quantified by GFAA or ICP. The respective detection limits are about 0.05 µg/g and 0.5 µg/g [assuming a 10 mg (dry weight) suspended matter sample is digested and the digestate is diluted to a final volume of 5.0 mL]. Chromium on intact filter media can also be determined by XRF spectroscopy. The detection limit of this technique is only about 50 µg/g, and the reproducibility is poor for natural chromium concentrations on suspended matter (approximately 50-150 µg/g). Note that chromium contamination of polycarbonate membrane filters can be very high. Strong-acid leaching with 12 N HCl for several days may be necessary to clean these filters sufficiently for chromium analysis.

Copper—Copper concentrations can be determined in freshwater samples by direct aspiration ICP analysis using a wavelength of 324.754 nm. The detection limit of this method is 6 µg/L, which meets the U.S. EPA freshwater chronic toxicity criterion. However, this method is not sensitive enough, by an order of magnitude, to detect copper in ambient fresh waters. With a 10-50X preconcentration, copper can be determined in most fresh waters by ICP analysis.

When copper is determined by GFAA using U.S. EPA Method 220.2 (Table 8), a detection limit of about 1 µg/L can be achieved. This sensitivity is often insufficient to quantify copper in ambient freshwater samples. Thus, a preconcentration method usually should be used. Many common preconcentration techniques (i.e., Chelex-100™, APDC/solvent extraction, Cobalt-APDC coprecipitation) will result in sufficient concentration factors to allow the accurate determination of copper in uncontaminated water samples (Appendix A).

Copper in suspended matter can be determined at a concentration of 0.2 µg/g when using GFAA or about 3 µg/g when using ICP. These detection limits assume that a 10 mg (dry weight) sample of suspended matter is acid-digested and the digestate is diluted to a volume of 5.0 mL prior to analysis. Typical suspended matter concentrations of copper are in the range of 10-100 µg/g. Copper in suspended matter may also be determined by XRF spectrometric analysis of the intact dry filter. However, the precision of XRF analysis may unacceptable for samples with small amounts of suspended material.

Iron—Iron concentrations can be determined by direct aspiration ICP analysis using an emission wavelength of 259.940 nm. The detection limit of 7 µg/L is often sufficient to accurately quantify iron in unfiltered and anoxic waters. Iron in virtually all natural fresh waters can be quantified using direct injection GFAA. This approach, using U.S. EPA Method 236.2 (Table 8), yields a detection limit of about 1 µg/L.

Although iron is present in natural water at relatively high concentrations, extreme care must be taken to avoid contamination. High concentrations of particulate iron occur on laboratory and terrestrial dust particles. It is necessary to rigorously implement clean-room techniques (e.g., filtered air, plastic-gloved hands, purified/analyzed reagents, and ultra-purity wash and dilution waters). It is good practice with iron analysis to run all samples in duplicate to help identify artificially high concentrations caused by sample contamination.

Fe^{+2} may be separately quantified by colorimetric determination of the ferrozine complex at neutral pH. Absorbance is measured at 562 nm using a 10-cm cell. The detection limit is about 0.5 µg/L. Fe^{+3} can be determined by difference from a total iron measurement by GFAA, or by colorimetric determination of a sample aliquot pre-reduced with hydroxylamine hydrochloride (Gibbs 1976).

A majority of the iron present in oxic fresh water is contained on the suspended matter, which can range from 0.5-10 percent iron on a dry weight basis. This fraction can easily be quantified on acid-digested filters by GFAA, ICP, or colorimetric analysis. In addition, iron on filter media is routinely analyzed by XRF spectrometry of the dry, intact filters. The filter pore size has a dramatic effect on the measurement of dissolved iron concentration because of the high concentrations of iron on colloids.

Lead—Lead concentrations in fresh waters cannot be quantified at either natural or U.S. EPA chronic toxicity criterion concentrations by any commonly used method except GFAA. Using the standard parameters from U.S. EPA Method 239.2 (Table 8), lead may be measured by direct injection at a concentration of about 1 µg/L. It is difficult to attain this detection limit unless the utmost care is taken to avoid sampling, laboratory, and reagent contamination. Very high concentrations of lead are associated with atmospheric particulate matter because of the combustion of leaded gasoline. It is necessary to rigorously implement clean-room techniques (e.g., filtered air, plastic-gloved hands, purified/analyzed reagents, and ultrapurity wash and dilution waters).

The reproducibility of lead analysis between samples is dramatically improved through the use of 2 percent $NH_4H_2PO_4$ as a matrix modifier. When matrix modification is used in conjunction with maximum-power atomization and the L'vov platform, a char temperature of 900° C and an atomization temperature of 2,200° C are optimal. The use of these refinements can result in a direct injection detection limit for lead as low as 0.2 µg/L.

To quantify lead in natural fresh waters, it is necessary to preconcentrate the sample prior to GFAA analysis. Several acceptable techniques are available for the 10-100X preconcentration of lead, which occurs in conjunction with other heavy metals. Acceptable preconcentration methods include ion-exchange, solvent extraction with a chelating agent, and chelate-metal coprecipitation (Appendix A). With the use of adequate clean-room techniques (e.g., Class 100), these methods can result in an ultimate detection limit of 0.01 µg/L or better. However, this sensitivity has been unattainable in most laboratories due to contamination.

Lead may be determined in particulate matter either by acid-digestion of the filter media followed by GFAA or ICP analysis, or by nondestructive XRF analysis of the intact filter. Assuming that a 10 mg (dry weight) suspended matter sample is digested and the digestate is diluted to a volume of 5.0 mL, the detection limit for GFAA is about 0.5 µg/g. With the same sample, a detection limit of 20 µg/g can be reached using ICP. This sensitivity is barely adequate for the determination of lead in particulate matter at the 50-200 µg/g levels commonly found in rural freshwater bodies. The detection limit using XRF is at least 5 µg/g for a similar quantity of particulate matter.

Manganese—Manganese concentrations in ambient freshwater samples can be determined by direct aspiration ICP analysis at 257.610 nm. This method has a detection limit of about 2 µg/L. This sensitivity is adequate for the quantification of manganese in most unpolluted fresh waters and in suspended matter digestates. For low-manganese waters, GFAA using the parameters of U.S. EPA Method 243.2 (Table 8) allows manganese detection to a concentration of about 0.5 µg/L. This detection limit, as well as intersample variability, may be improved upon by as much as a factor of 10 by using 0.5 percent $Mg(NO_3)_2$ as a matrix modifier. When using the matrix modification in conjunction with an instrument capable of maximum power atomization, a char temperature of 1,200° C and atomization temperature of 2,400° C are optimal.

Concentrations of manganese bound to suspended matter may be readily determined by either ICP or GFAA analysis of acid-digested filters. Typical freshwater particulate manganese concentrations are 100-1,000 µg/g, which is well above the detection limit of either technique. Manganese may also be quantified by XRF spectroscopy of the intact filter media.

Mercury—The only commonly available method suitable for the determination of mercury concentrations in ambient freshwater samples is by reduction to elemental mercury, followed by purging with carrier gas into one of several types of atomic absorption spectrometers. Mercury is commonly reduced to its gaseous form using $SnCl_2$ (e.g., U.S. EPA Methods 245.1 and 245.2) or $NaBH_4$. The mercury vapor may be directly analyzed as it is purged. It may also be pretrapped by amalgamation on a gold substrate prior to analysis. The most common method for detection is CVAA operating at the 254-nm resonance line. Using a 100 mL sample and purging directly into the CVAA detector (U.S. EPA Method 245.1), a detection limit as low as 0.2 µg/L can be reached. This detection limit is too high to measure mercury at the U.S. EPA freshwater chronic toxicity criterion concentration, and it is orders of magnitude too high to measure ambient concentrations.

Mercury that is purged from the sample must be preconcentrated prior to analysis. Preconcentration is done by passing the gas stream over gold foil (Bloom and Crecelius 1983) or gold-coated sand (Fitzgerald and Gill 1979). The gold amalgamates with the mercury, while allowing the gas stream and entrained water vapor to pass through. The mercury on the gold trap is then thermally desorbed at 300° C into the CVAA detector for analysis. This procedure dramatically increases the sensitivity of the method, reducing the detection limit for 100 mL samples to approximately 0.0005 μg/L when using CVAA or 0.00005 μg/L when using cold vapor atomic fluorescence (Bloom and Fitzgerald in press).

The majority of mercury in freshwater samples is bound with organic materials. Thus, it is not subject to volatilization with $SnCl_2$ addition without prior oxidation to break down the organomercury bonds. This oxidation may be achieved using reagents such as bromine monochloride, potassium persulfate, $KMnO_4/HCl$, and H_2SO_4/K_2CrO_4. Ultraviolet photo-oxidation may also be used. The potential for contamination increases substantially with these additional steps.

Although the gold trapping/atomic spectroscopy methods are simple, accurate, and sensitive, far greater handling care must be taken with mercury than with any other element. This care is necessary because of mercury's low natural concentrations, ubiquity in the laboratory, and presence as a gaseous component of the air. Thus, clean-room techniques involving laboratory air, plastic-gloved hands, and purity of reagents and water must be rigorously implemented. Polyethylene, polypropylene, vinyl, and silicone containers and tubing are all incompatible with mercury sampling and analysis because they are porous to gaseous atmospheric mercury. The only truly suitable materials for mercury analysis are rigorously acid-cleaned fluoropolymer, glass, and quartz. In short contact-time situations, acid-cleaned polystyrene, acrylic, and polycarbonate plastics can be used.

All air and gases used in purging and analysis must be passed through gold columns just prior to use. The purpose of this step is to eliminate the often large concentrations of entrained mercury. Laboratory water is often contaminated with mercury at concentrations far higher than those of the natural environment. Typical deionized or distilled laboratory water contains mercury at concentrations from 0.01-10 μg/L. This range is 1-4 orders of magnitude greater than the highest expected concentration in ambient fresh water. Ordinary deionized water is commonly contaminated with mercury because the industrial-grade sodium hydroxide used to recharge the anion exchange beds is often made using a mercury electrode process.

The only waters acceptable for equipment clean-up and dilutions during mercury analysis are super-high-purity laboratory grade deionized water, sub-boiling double distilled water from a clean quartz or fluoropolymer still, and continuously running tap water. The latter, especially if its source is a deep well, is generally the water with the lowest mercury concentration, typically containing as little as 0.0001 μg/L. However, tap water can generally not be used for clean-up or dilution with any metal except mercury.

Acids used to preserve samples intended for mercury analysis should be selected from previously analyzed case-lots of reagent-grade material stored in borosilicate glass bottles. The acid selected should be found sufficiently low in mercury (i.e., <0.10 μg/L) to meet the desired limits of detection when 1,000X dilution in the sample is taken into account. Low mercury acids can also be prepared by repeated distillations in an all-fluoropolymer sub-boiling still. The commercial ultrapure acids should be avoided because they often contain orders of magnitude higher mercury concentrations than do ordinary reagent-grade acids. Stannous chloride, the most commonly used reagent for the conversion of ionic mercury to volatile mercury, may be purified by bubbling the reagent solution overnight with a mercury-free inert gas.

Special care should be taken to tighten sample containers completely in the field, and then to analyze the samples for mercury immediately after the sample containers are opened. Laboratory air often contains very high concentrations of gaseous mercury, which can diffuse into the acidified sample through loose or open caps. The best approach to minimize potential mercury contamination in the laboratory is to increase the flushing rate of the room air with air from outside. Ambient air generally has a very low mercury concentration. Also, mercury-containing equipment (e.g., polarographic equipment, barometers, thermometers) must not be allowed in the room in which mercury analysis takes place.

Mercury may be analyzed in suspended matter by digestion of the wet filter in BrCl or aqua regia, followed by $SnCl_2$ reduction, purging onto gold, and CVAA detection. Because the digestion blanks are typically high, detection limits using this method are often in the range of 0.1 µg/g. The established methods that currently exist for filtering unpolluted water for mercury analysis all have the potential for gross contamination of the filtrate. Thus, analysis of filtered waters for mercury is not recommended.

Nickel—Nickel concentrations in freshwater samples can be determined by direct aspiration ICP analysis at 231.604 nm. This method has a detection limit of about 15 µg/L. This sensitivity is adequate for determining compliance with the U.S. EPA freshwater chronic toxicity criterion of 160 µg/L, but it is not adequate for nickel determination in natural samples (Table 7). The detection limit for nickel in fresh waters by GFAA, using U.S. EPA Method 249.2, is about 1 µg/L. This sensitivity is also generally inadequate for the quantification of ambient nickel concentrations.

A preconcentration technique such as chelate/solvent extraction, ion exchange, or cobalt-APDC coprecipitation must be used for determination of ambient nickel concentrations (Appendix A). If the latter method is employed, it is critical that only ultra-high purity (electronics grade) cobalt be used in preparing the reagent. Laboratory-grade cobalt salts contain unacceptably high concentrations of nickel. If high-purity cobalt is unavailable, an alternative approach is to use another metal, (e.g., mercury) in the place of the cobalt for the preconcentration of nickel by the coprecipitation (Boyle and Edmond 1975; Bloom and Crecelius 1984).

When nickel-free reagents are used, high concentration factors with low blank concentrations are easily attainable, giving final detection limits for nickel in the range of 0.01 µg/L. Matrix modification is generally not required for the analysis of nickel by GFAA, although the standard $NH_4H_2PO_4$ and $Mg(NO_3)_2$ modifiers used for other metals are not detrimental to nickel analyses. The use of pyrolytic graphite in the atomization tube is strongly recommended because of the formation of refractory carbides when ordinary graphite is used.

Nickel can be easily determined in particulate matter either by HF/aqua regia digestion of the filters followed by GFAA analysis, or by non-destructive XRF analysis of the intact filters. Assuming a 10 mg (dry weight) suspended matter sample is digested and the digestate is diluted to a final volume of 5.0 mL, the detection limit for GFAA is about 0.5 µg/g, and the detection limit for XRF is about 20 µg/g. Typical concentrations of nickel in suspended matter are about 50-100 µg/g.

Silver—ICP analysis is inadequate for the determination of silver, even in the most polluted waters. The detection limit of 20 µg/L is 2 orders of magnitude too high to meet the U.S. EPA chronic toxicity criterion (0.12 µg/L) (see Table 7), and is approximately 4 orders of magnitude above natural concentrations. Direct injection GFAA, using U.S. EPA Method 272.2 (Table 8) can just meet the chronic toxicity criterion, but is still not sensitive enough for environmental measurements. The detection limit for direct injection GFAA can be improved to about 0.05 µg/L through the use of 2 percent $NH_4H_2PO_4$ as a matrix modifier and the L'vov atomization with maximum power. If these techniques are used, the char temperature may be increased to 800° C and the atomization temperature reduced to 2,000° C (Table 8). Pyrolytic graphite tubes and platforms should be used, and advanced background correction is recommended. Using the above modifications, silver concentrations in reagent and handling blanks are very low, making the lowest detection limits readily attainable.

To quantify the exceedingly low ambient silver concentrations found in natural waters, preconcentration of 20-100X is necessary. The most simple and economical technique for this purpose is the cobalt-APDC coprecipitation method (Bloom and Crecelius 1984). This method has been optimized for silver to yield a detection limit of approximately 0.0002 µg/L using a 100X preconcentration and GFAA detection (see Appendix A).

The only readily available method for the determination of silver in suspended matter is GFAA analysis of the digested filter media. Assuming 10 mg (dry weight) of suspended matter is dissolved into 5.0-mL final solution volume, the detection limit of this technique is about 0.05 µg/g. This sensitivity is adequate to determine ambient suspended matter concentrations, which are typically 0.1-2.0 µg/g. The use of a mixed matrix-modifier containing 2 percent $NH_4H_2PO_4$ plus 0.5 percent $Mg(NO_3)_2$ and advanced background correction is essential to obtaining good results for silver in particulate matter. The analytical system should be calibrated by the method of standard additions.

Zinc—Direct aspiration ICP analysis at 213.856 nm can be used for the crude determination of zinc in uncontaminated, freshwater samples. The detection limit of 7 µg/L is approximately at the concentration observed in such waters. GFAA will easily yield accurate results for zinc in ambient freshwater samples, if sufficient care is taken to avoid field and laboratory contamination of the samples. Contamination by zinc, like that of lead, iron, and mercury, is almost impossible to control without the rigorous implementation of clean-room techniques (e.g., filtered air, plastic-gloved hands, purified/analyzed reagents, and ultra-high purity wash and dilution waters). When these conditions are met, zinc concentrations can be reliably determined at about 0.05 µg/L using U.S. EPA Method 289.2 (Table 8). The common practice of pulling and cutting the pipette tip on Perkin-Elmer's autosampler AS-40 is discouraged since this action breaks the seal and causes zinc to leak into the matrix modifier and sample.

Intersample reproducibility is greatly enhanced through the use of 2 percent $NH_4H_2PO_4$ as a matrix modifier. The phosphate solution must be passed through an ion-exchange column or solvent extracted with an appropriate chelating agent (i.e., APDC, dithiazone) to remove all traces of zinc. Zinc contamination is so ubiquitous that even disposable auto-sampler cups used with the GFAA system should be individually acid cleaned, rinsed with ultrapure water, and then rinsed several times with the sample to be analyzed, prior to use. It is good practice with zinc analysis to run all samples in duplicate to help identify artificially high concentrations caused by sample contamination.

Zinc concentrations in particulate matter may be determined either by acid digestion of the filter media followed by GFAA or ICP analysis, or by nondestructive XRF analysis of the whole filter. In either case, great care must be exercised in sample handling and reagent purity. Total handling blanks should be run often. The detection limit for zinc analyzed by acid digestion followed by spectroscopic analysis is about 1-10 µg/g, depending upon concentrations found in the blanks. All of these methods will generally result in accurate results for zinc concentrations in suspended matter, which usually are in the range of 100-200 µg/g.

QA/QC PROCEDURES

QA/QC measures must be applied over the entire data collection process, from instrument calibration and method evaluation to field sampling and data archiving. In all cases, at least the minimum level of QA/QC should be applied, as required by the U.S. EPA in its Contract Laboratories Program Statement of Work (U.S. EPA 1987a). Refer to the PSEP protocols document on metals (PSEP 1989) for a detailed description of appropriate QA/QC measures.

At a minimum, laboratories should perform the quality control checks listed below:

- Method (or procedural) blank analysis
- Spiked sample analysis
- Replicate sample analysis
- GFAA method of standard addition (if necessary)
- Certified reference materials analysis, (e.g., SLRS-1 riverine water for trace metals, available from National Research Council of Canada Standards Program, Ottawa, Canada).

DATA REPORTING REQUIREMENTS

Sample results should be reported after the method blank has been subtracted. The method blank must also be reported for each batch of samples. Dissolved metals are reported in units of µg/L. Particulate metals are reported in units of µg/g dry weight. Refer to the PSEP protocols document on metals (PSEP 1989) for a detailed description of data reporting requirements.

The method detection limit for an element must be less than or equal to the required detection limit for that element. Required detection limits are determined by the needs of the individual project and must be specified contractually.

The method detection limit for an element is calculated as 3 times the standard deviation of the concentrations of that element found in the method blanks. At least three method blanks should be analyzed in order to calculate the detection limit; pooling duplicate blank results from a series of sample batches will provide a superior means of estimating the detection limit. When the concentrations of metals in samples are equal to or less than the detection limit after the method blank is subtracted, the "less than" symbol (<) should be entered together with the detection limit in the data report.

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APPENDIX A

A CHELATE-COPRECIPITATION METHOD
(Bloom and Crecelius 1984)

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DETERMINATION OF SILVER IN SEA WATER BY COPRECIPITATION WITH COBALT PYRROLIDINEDITHIOCARBAMATE AND ZEEMAN GRAPHITE-FURNACE ATOMIC ABSORPTION SPECTROMETRY

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SUMMARY

A preconcentration technique is described for silver, which allows the precise and accurate determination of silver in sea water at nanogram per liter levels. Sliver is coprecipitated with cobalt(II) pyrrolidinedithiocarbamate from 200-ml samples. The precipitate is dissolved in concentrated nitric acid and silver is quantified by Zeeman graphite-furnace atomic absorption spectrometry, with acid phosphate matrix modification. The detection limit is 0.1ng 1^{-1}. The method is simple and rapid, and also allows the simultaneous extraction of Iead, copper, cadmium and nickel.

In view of the possible toxicity [1-3] and enrichment [4-6] of silver in the marine environment, it is surprising that so little work has been done on establishing the concentration of silver in sea water. Earlier attempts to quantify silver in sea water have typically led to high values [7], inadequate detection limits [8], or noisy data. In response to the need for a simple and reliable method for accurately quantifying silver in sea water, the following technique was developed. The method represents a refinement of the coprecipitation procedure based on cobalt ions and ammonium pyrrolidinedithiocarbamate (APDC), which was used by Boyle and Edmond [9,10] to determine copper, cadmium and nickel. The extraction parameters were optimized for silver using a radiotracer. Silver was quantified by graphite-furnace Zeeman atomic absorption spectrometry (a.a.s.). Several other metals, including lead, copper, cadmium, and nickel are co-extracted with silver, making the procedure economical for multielement quantitation.

EXPERIMENTAL

Reagents and solutions

Water used for the rinsing of glassware was deionized to a minimum of one megohm resistance. For reagent preparation and sample dilution, double-deionized water from a Millipore Super-Q reagent-water system (18 megohms resistance) was used. Acid used in the washing of glass and plasticware was 6 M nitric acid (reagent grade). Ultra-high purity nitric acid was used in the preparation of reagents and samples.

Cobalt(II) nitrate solution. A solution containing 200 mg 1^{-1} cobalt(II) was prepared by dissolving 200 mg of ultrapure cobalt wire (Ventron) in 5 ml of nitric acid, and diluting to 1 l with water.

Ammonium pyrrolidinedithiocarbamate (APDC) solution. A 2% (w/v) solution of APDC in water was purified by repeated extraction with carbon tetrachloride [10] . This solution was stored in a refrigerator when not in use.

Ammonium acetate buffer. A 4.5 M solution of ammonium acetate was prepared by the reaction of 500 ml of ultra-high purity acetic acid with 590 ml of ultra-high purity aqueous ammonia (28%), and dilution to 2.0 l with water.

Extract diluting solution. The digested precipitates were diluted to volume with a solution containing 0.2% (w/v) high-purity ammonium dihydrogenphosphate and 5% (v/v) nitric acid.

^{110m}Ag tracer. A stock solution containing 40 µCi ml-^{1} ^{110m}Ag and 9.4 mg l-^{1} total silver was prepared by diluting 2 mCi of ^{110m}Ag into 50 ml of 5% nitric acid. A working solution was prepared from this by diluting 2 ml of the stock to 100 ml with 5% nitric acid.

Acid-cleaned filters. The precipitate of cobalt with APDC was filtered through acid-cleaned, 47-mm, 0.4-µm polycarbonate membrane filters. The filters were always handled with acid-cleaned fluorinated polyethylene tongs.

Vials. The filters were digested and the solutions were diluted to volume in acid-cleaned 17-ml fluorinated polyethylene wide-mouth vials (Savillex, Minetonka, MN).

Instrumentation

Atomic absorption measurements were done with a Perkin-Elmer model Z-5000 graphite-furnace atomic absorption spectrometer, with Zeeman background correction. This system includes the HGA-500 furnace controller, Model 10 data station with HGA graphics and the AS-40 autosampler. The operating parameters are listed in Table 1. A hollow-cathode silver lamp was used, and the monochromator was set at 328.1 nm (0.7-nm slit width).

Radioactive decay of the ^{110m}Ag was counted on a Princeton Gamma-tech Ge(Li) detector, using the 658-keV photon peak. Peak separation and quantitation were achieved with a Canberra series 40 multichannel analyzer.

Preliminary work

Sea-water storage. Experiments to evaluate losses of silver to container walls during storage were done at pH values of 8.1, 1.9, and 1.6. Fresh, filtered sea water was placed in 2-l polyethylene bottles. The water in each bottle was brought to the desired pH with nitric acid, and then spiked with ^{110m}Ag equal to 1.0 µg l-^{1} total Ag. The bottles were stored at room temperature, with lights on for approximately 12 h per day.

TABLE 1

Furnace controller parameters for the graphite-furnace a.a.s. measurement of silver in seawater extracts[a]

Step number	Ramp time (s)	Hold time (s)	Temperature (□C)	Gas flow	Recorder	Zeeman
1	10	0	80	300	off	off
2	50	5	130	300	off	off
3	25	5	250	300	off	off
4	20	15	-150	300	off	off
5	0	3	1800b	0	on	on
6	1	4	2600	300	off	off

[a] Pyrolytic graphite cylindrical tube, argon purge gas, 25-µl sample.
[b] Maximum-power mode of the Perkin-Elmer HGA-500.

Optimization of extraction parameters. The parameters were optimized using 100-ml aliquots of sea water (about 30% salinity) that had been spiked with ^{110m}Ag to contain 1.0 µg I-^{1} total silver. The stock of this solution was kept acidified to pH 1.70, with pH adjustments being made on individual aliquots using the ammonium acetate buffer.

The coprecipitation reactions were carried out in 150-ml beakers, by the addition first of the cobalt solution, thorough mixing, and then addition of a 100-fold excess (mass basis) of APDC. Precipitates were vacuum-filtered through 0.4-µm membrane filters. The filters were placed in 60-ml polyethylene bottles with 5.0 ml of nitric acid to digest the precipitate, and then filled with water. The radioactive decay of these samples was counted to determine total silver recovered.

Recommended procedures

Sea-water samples were collected on the beach or offshore in 2-l polyethylene bottles. The samples are immediately acidified with 2.10 ± 0.05 ml of concentrated nitric acid; this brings the pH to 1.90 ± 0.05. In the laboratory, an aliquot of the sample is transferred to a 250-ml volumetric flask.

From each 250-ml sample, 50 ml is poured off and discarded, leaving 200 ml in the flask which serves as the reaction vessel. To each 200-ml sample is added 1.0 ml of cobalt(II) solution and 1.0 ml of APDC solution, with swirling after each addition to insure thorough mixing.

The samples are then filtered by suction through 47-mm acid-cleaned polycarbonate membrane filters (0.4 µm). The filters are rinsed with about 10 ml of water to remove salts, then folded into quarters, and placed in clean, dry 17-ml vials. To each filter is added 210 µl of concentrated nitric acid and the samples are evaporated to dryness under heat lamps. This digestion destroys the APDC complex, but leaves the polycarbonate filters intact, thus minimizing organic matrix interference in the atomic absorption measurements.

Reagent blanks are prepared by adding one filter, 1.0 ml of cobalt(II) solution, and 1.0 ml of APDC to a vial, and bringing to dryness, as for the samples. The blanks are digested with 420 µl of nitric acid, which represents the amount used to acidify the sea-water samples plus the amount used to digest the sample precipitates.

To the dried filters in the vials, 2.00 ml of the phosphate diluting solution are added. The lids are replaced and the samples are gently heated (70-80°C) to dissolve the metals on the filter. The samples are then stored in the vials until quantitation by graphite-furnace a.a.s. as outlined above.

RESULTS AND DISCUSSION

Sea-water storage conditions

As can be seen *in* Fig. 1, silver was not lost to container walls from sea water at pH < 2.0 whereas losses from unacidified sea water were significant. This confirms the work of Massee et al. [11] , Struempler's observation [12] that in distilled water, even acidified samples need shielding from light, does not appear to be true for sea water. It should be noted that, because of the low specific activity of the ^{110m}Ag tracer, it was necessary to work at total silver concentrations 1000-fold those of clean sea water. Thus, these results must be applied tentatively, until a carrier-free radiotracer can be obtained.

Optimization of extraction parameters

Because Boyle and Edmond [10] indicated that pH was the most important parameter for the extraction of several metals (Cd, Fe, Mn), this was the first condition to be optimized for silver. Recovery of ^{110m}Ag was monitored over a range of pH values from 1.70 to 5.50. These data, expressed as percent silver recovered, are illustrated in Fig. 2. At very low pH, the yield drops off, with a maximum Yield at pH 1.8-2.0, followed by a rapid decrease with increasing pH.

Figure 1

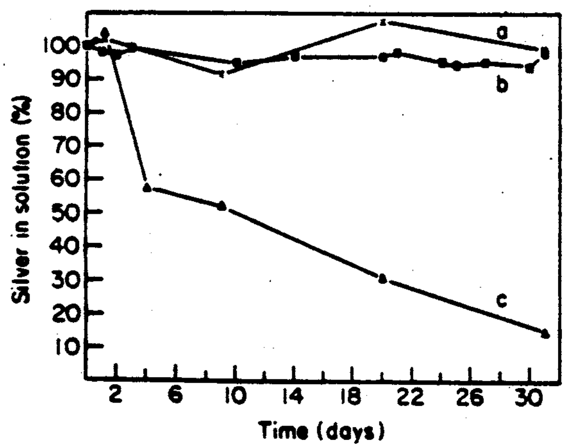

Figure 2

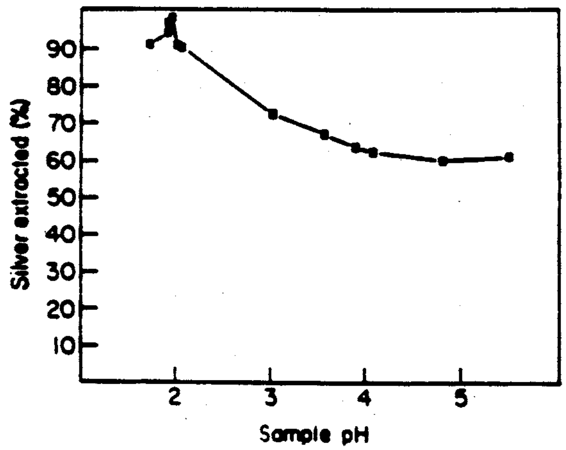

Figure 1. Loss of ^{110m}Ag from sea water to the walls of acid-cleaned polyethylene sampling bottles as a function of time at different pH values: (a) pH 1.60; (b) pH 1.90; pH 8.10. Total silver concentration in each case was 1.0 µg 1^{-1}.

Figure 2. Extraction efficiency for ^{110m}Ag by the Co-APDC method, as a function of sample pH. Total silver concentration was 1.0 µg 1^{-1}.

The minimization of the amount of extraction reagents was also examined because of their potential contribution to the blank. Samples of filtered sea water, at pH 1.90, were spiked with silver (1.0 µg 1-^{1}). Varying amounts of cobalt(II) ranging from 0.05 to 1.0 mg l-^{1} were added, along with a proportional 100-fold excess of APDC. The recoveries of silver increased sharply to about 88% as the amount of cobalt(II) was increased to 0.25 mg l-^{1} and then increased gradually to 92% at a cobalt(II) concentration of 1.0 mg l-^{1}. This concentration gave reproducible results (± 10%) without admitting excessive amounts of reagents.

The other factors investigated in the optimization of this procedure were: (a) the time of precipitate ageing, from 5 min to 8 h; and (b) the rinse volume for the filters, from 0 to 100 ml of purified water. Neither of these factors influenced the yield of silver recovery to an observable extent.

Radiotracer experiments were also undertaken to ascertain whether the extraction technique was suitable for use over a wide range of silver concentrations. Samples were prepared in filtered Sequim Bay water containing 1.00, 0.1, and 0.010 µg 1-^{1} total silver. These were then extracted using the optimized parameters described above. The results showed consistent yields of 92-95% for all concentrations of silver in sea water.

Atomic absorption parameters

With the use of the 0.2% (w/v) ammonium phosphate matrix sample diluting solution, problems of variable peak shape and multiple peaks are eliminated. The conditions given in Table 1 represent the optimum in terms of precision, accuracy, and graphite tube lifetime for the Perkin-Elmer Z-5000 system. The length of the drying stage is dependent upon the sample volume, and is given for a 25-µl aliquot. Care must be taken that the drying ramp time is not too short as the phosphate matrix is quite prone to spattering at 120-140°C. The 1800°C atomization temperature is made possible by the maximum-power mode of the furnace controller. For an ordinary atomization step, a temperature of 2400°C would be appropriate for this matrix.

In this work all measurements were done using long-lasting pyrolytic graphite tubes, prepurified argon purge gas, and Zeeman background correction. Under the conditions recommended and with this matrix, repetitive firings of the same sample varied by less than 5%. The linear range was well over 1.0 absorbance. All standards were run by the method of standard additions on the blanks. Under such conditions, a 25-µl sample containing 10 µg l^{-1} silver gave an absorbance of approximately 0.700.

Application, precision and detection limits

Table 2 summarizes typical results of the complete procedure. The samples were made from clean, unfiltered water from Sequim Bay, Washington. A sample of the water was acidified to pH 1.90 ± 0.05, and divided into three aliquots. One aliquot was spiked with 0.01 µg l^{-1} silver and another with 0.1 µg l^{-1} silver. All three aliquots were then allowed to equilibrate overnight before extraction.

TABLE 2

Quantitation of silver in spiked sea water by the proposed method

Sample	N	Ag (ng l^{-1})[a] x	s	Yield(%)
Blanks	5	0.11	0.04	--
Sequim Bay Water	3[b]	0.63	0.12	--
SBW+10 ng l^{-1}	5	10.74	0.29	99.8
SBW+100 ng l^{-1}	4	94.0	7.9	93.2

[a]Mean of *N* measurements with standard deviation. Two obviously contaminated values of 5.94 and 1.57 were omitted.

The detection limit is 0.2 ng l^{-1} based on twice the standard deviation of samples near the detection limit. If twice the standard deviation of the blank is taken as the criterion, the detection limit is 0.1 ng l^{-1}. At all levels measured, more than 90% of the silver was recovered with a precision of better than 10%.

Environmental samples. Samples from a variety of Pacific Northwest locations were processed as described above. The data for some of these are summarized in Table 3. Three types of coastal water, as well as the open ocean, are represented in these data. Sequim Bay is a rural area on the well flushed Strait of Juan de Fuca, Elliott Bay is the major harbor for the city of Seattle, and the marina in Tacoma represents a unique local environment, as it is built with slag from a copper smelter. The three depth "profiles" taken in the northeast Pacific shows an increase in silver with depth from about 0.3 ng 1^{-1} to 5.8 ng 1^{-1}. This trend is essentially identical to two other profiles taken in the same area in the last two years by Battelle.

TABLE 3

Silver concentrations in selected marine samples

Date	Location	Depth	*N*	Silver (ng 1-^{1}) $\bar{x}$	*s*
11-23-82	Sequim Bay	10 cm	3	0.63	0.12
9-13-82	Elliott Bay	10 cm	2	10.0	--
1-6-82	Tacoma (Marina)	10 cm	2	108.0	--
1-20-82	Tacoma (Marina)	10 cm	1	19.0	--
3-3-82	Tacoma (Marina)	10 cm	2	13.5	--
2-12-82	N.E. Pacific Ocean	50 m	3	0.30	0.10
2-12-82	N.E. Pacific Ocean	300 m	2	0.60	--
2-13-82	N.E. Pacific Ocean	3237 m2	5.82	--	

The recent work of Martin and Knauer (personal communication) in the northeast Pacific ocean shows a similar trend for a more detailed profile over the same range.

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APPENDIX B

A VOLATILIZATION METHOD BY HYDRIDE GENERATION
(Crecelius et al. 1986)

Speciation of Selenium and Arsenic in Natural Waters and Sediments

Volume 2: Arsenic Speciation

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Section 2

DETERMINATION OF ARSENIC SPECIES IN LIMNOLOGICAL SAMPLES BY HYDRIDE GENERATION ATOMIC ABSORPTION SPECTROSCOPY

INTRODUCTION

This section describes the analytical methods used to determine the arsenic species in waters and sediments. Also, sample storage tests were conducted to select methods of storing and shipping environmental samples that would minimize changes in speciation. Based on results of previous studies we selected hydride generation coupled with atomic absorption spectroscopy as the method of quantification of arsenic. In this technique arsenate, arsenite, methylarsonic acid, and dimethylarsinic acid are volatilized from solution at a specific pH after reduction to the corresponding arsines with sodium borohydride (1). The volatilized arsines are then swept onto a liquid nitrogen cooled chromatographic trap, which upon warming, allows for a separation of species based on boiling points. The released arsines are swept by helium carrier gas into a quartz cuvette burner cell (2), where they are decomposed to atomic arsenic. Arsenic concentrations are determined by atomic absorption spectroscopy. Strictly speaking, this technique does not determine the species of inorganic arsenic but rather the valence states of arsenate (V) and arsenite (III). The actual species of inorganic arsenic are assumed to those predicted by the geochemical equilibrium model described in Section 1 of this report.

EXPERIMENTAL SECTION

Apparatus.

The apparatus needed for the volatilization, separation and quantitation of arsenic species is shown schematically in Figure 2-1-a. Briefly, it consists of a reaction vessel, in which arsenic compounds are reduced to volatile arsines, a liquid nitrogen cooled gas chromatographic trap, and a H_2 flame atomic absorption detector.

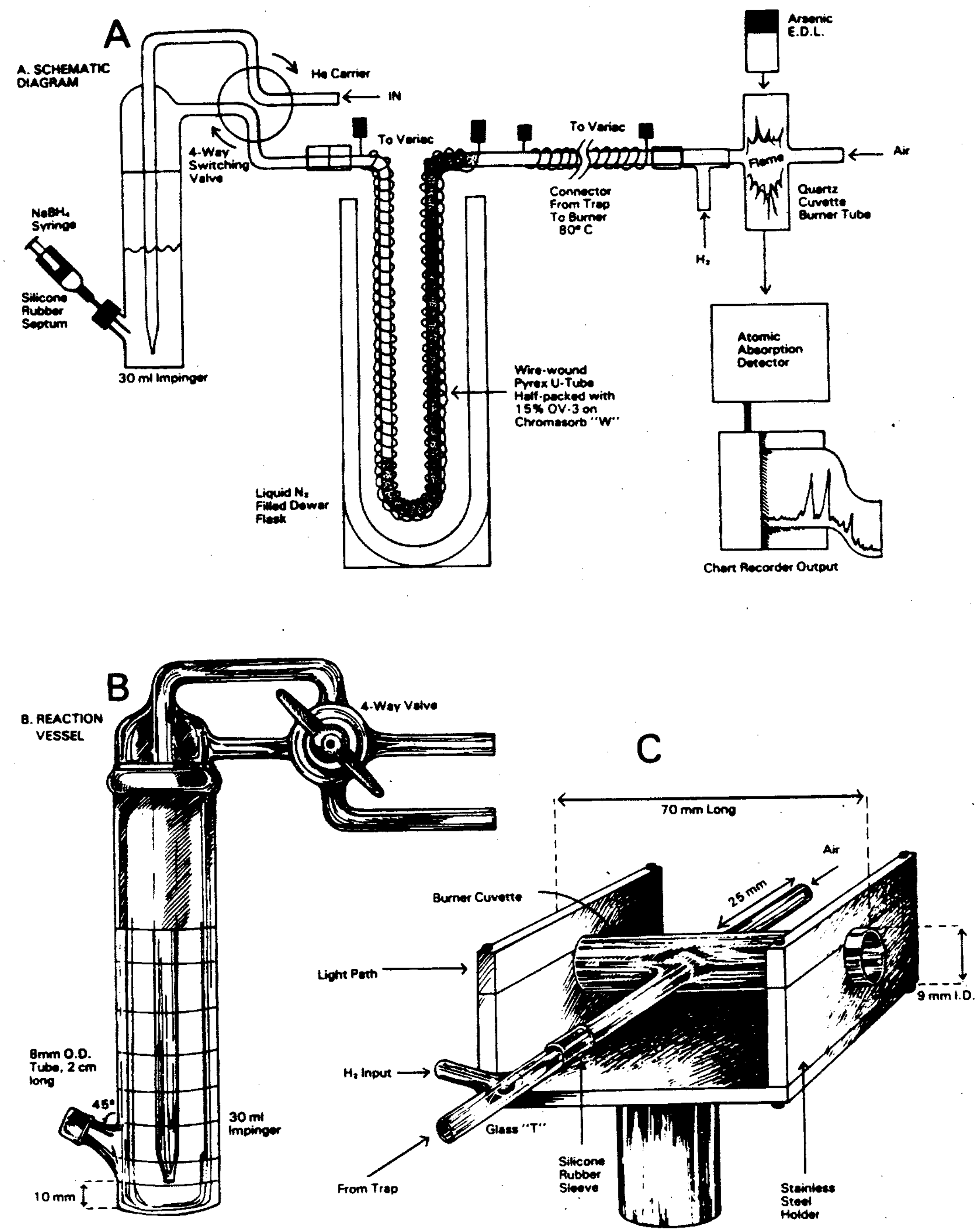


Figure 2-1. Arsenic Speciation Apparatus: (a) Schematic Diagram; (b) Reaction Vessel; (c) Quartz Cuvette Burner Tube.

Reaction Vessel. The reaction vessel is made by grafting a side-arm inlet onto a 30-ml "Midget Impinger" (Ace Glass #7532-20), as illustrated in Figure 2-1-b. The 8-mm diameter side arm may then be sealed with a silicone rubber-stopper type septum (Ace Glass 09096-32) to allow the air-free injection of sodium borohydride. The standard impinger assembly is replaced with a 4-way Teflon stopcock impinger (Laboratory Data control #700542) to allow rapid and convenient switching of the helium from the purge to the analysis mode of operation.

GC Trap. The low temperature GC trap is constructed from a 6 mm o.d. borosilicate glass U-tube about 30-cm long with a 2-cm radius of band (or similar dimensions to fit into a tall widemouth Dewar flask. Before packing the trap, it is silanized to reduce the number of active adsorption sites on the glass. This is accomplished using a standard glass silanizing compound such as Sylon-Ct®(Supelco Inc.). The column is half-packed with 15% OV-3 on Chromasorb® WAW-DMCS (45-60 mesh). A finer mesh size should not be used, as the restriction of the gas flow is sufficient to overpressurize the system. After packing, the ends of the trap are plugged with silanized glass wool.The entire trap assembly is then preconditioned as follows: The input side of the trap (non-packed side) is connected via silicone rubber tubing to helium at a flow rate of 40 ml•min^{-1} and the whole assembly is placed into an oven at 175°C for 2 hours. After this time, two 25-µl aliquots of GC column conditioner (Silyl-8®, Supelco Inc.) are injected by syringe through the silicone tubing into the glass tubing. The column is then left in the oven with helium flowing through it for 24 hours. This process, which further neutralizes active adsorption sites and purges the system of foreign volatiles, may be repeated whenever analate peaks are observed to show broadening.

Once the column is conditioned, it is evenly wrapped with about 1.8 m of nichrome wire (22 gauge) the ends of which are affixed to crimp on electrical contacts. The wire-wrapped column is then coated about 2-mm thick all over with silicone rubber caulking compound and allowed to dry overnight. The silicone rubber provides an insulating layer which enhances peak separation by providing a longer temperature ramp time.

The unpacked side of the column is connected via silicone rubber tubing to the output from the reaction vessel. The output side of the trap is connected by a nichrome-wire wrapped piece of 6-mm diameter borosilicate tubing to the input of the flame atomizer. It is very important that the system be heated everywhere (~80°C) from the trap to the atomizer to avoid the condensation of water. Such condensation can interfere with the determination of dimethylarsine. All glass-to-glass connections in the system are made with silicone rubber sleeves.

Atomizer. The eluted arsines are detected by flame atomic absorption, using a special atomizer designed by Andreae (2). This consists of a quartz cross tube as shown in Figure 2-1-c. Air is admitted into one of the 6-mm o.d. side tubes (optimal flows are given in Table 2-1), while a mixture of hydrogen and the carrier gas from the trap is admitted into the other. This configuration is superior to that in which the carrier gas is mixed with the air (Andreae, personal communication 1983) due to the reduction of flame noise and possible extinguishing of the flame by microexplosions when H_2 is generated in the reaction vessel. To light the flame, all of the gases are turned on, and a flame brought to the ends of the quartz cuvette. At this point a flame will be burning out of the ends of the tube. After allowing the quartz tube to heat up (~5 minutes) a flat metal spatula is put smoothly first over one end of the tube, and then the other. An invisible air/hydrogen flame should now be burning in the center of the cuvette. This may be checked by placing a mirror near the tube ends and checking for water condensation. Note that the flame must be burning only inside the cuvette for precise, noise-free operation of the detector.

Table 2-1

OPTIMAL FLOWS AND PRESSURES FOR GASES IN THE HYDRIDE GENERATION SYSTEM

Gas	Flow rate ml•min-^{1}	Pressure Ib•in-^{2}
He	150	10
H_2	350	20
Air	180	20

Precision and sensitivity are affected by the gas flow rates and these must be individually optimized for each system, using the figures in Table 2-1 as an initial guide. We have observed that as the $0_2/H_2$ ratio goes up, the sensitivity increases and the precision decreases. As this system is inherently very sensitive, adjustments are made to maximize precision.

Detector. Any atomic absorption unit may serve as a detector, once a bracket has been built to hold the quartz cuvette burner in the wave path. This work has been done using a Perkin-Elmer Model 5000® spectrophotometer with electrodeless discharge arsenic lamp. An analytical wavelength of 197.3 nm and slit width of 0.7 nm (low) are used throughout. This wavelength has been shown to have a longer linear range, though about half the sensitivity of the 193.7 nm line (2). Background correction is not used as it increases the system noise and has never been found necessary on the types of sample discussed in this paper.

Standards and Reagents

Arsenite (As(III)) Standards. A 1000 $mg \cdot l^{-1}$ stock solution is made up by the dissolution of 1.73 grams of reagent grade $NaAsO_2$ in 1.0-liter deionized water containing 0.1% ascorbic acid. This solution is kept refrigerated in an amber bottle. A 1.0 $mg \cdot l^{-1}$ working stock solution is made by dilution with 0.1% ascorbic acid solution and stored as above. Under these conditions this solution has been found stable for at least one year.

Further dilutions of As(III) for analysis, or of samples to be analyzed for As(III), are made in filtered Dungeness River water. It has been observed both here and elsewhere (Andreae 1983) that deionized water can have an oxidizing potential that causes a diminished AS(III) response at low levels (1 $mg \cdot l^{-1}$ and less). Dilute As(III) standards are prepared daily.

Arsenate (As(V)) Standards. To prepare a 1,000 $mg \cdot l^{-1}$ stock solution, 4.16 g of reagent grade $Na_2HASO_4 \cdot 7H_2O$ are dissolved in 1.0 liter of deionized water. Working standards are prepared by serial dilution with deionized water and prepared monthly.

Monomethylarsonate (MMA) Standards. To prepare a stock solution of 1000 $mg \cdot l^{-1}$, 3.90 g of $CH_3AsO(ONa)_2.6H_2O$ is dissolved in 1.O liter of deionized water. Working standards are prepared by serial dilution with deionized water. Dilute standards are prepared weekly.

Dimethylarsinate (DMA) Standards. To prepare a stock solution of 1,000 $mg \cdot l^{-1}$, 2.86 g of reagent grade $(CH_3)_2AsO_2Na \cdot 3H_2O$ (cacodylic acid, sodium salt) is dissolved in 1.0 liter deionized water. Dilute standards are handled as for MMA.

6M Hydrochloric Acid. Equal volumes of reagent grade concentrated HCI and deionized water are combined to give a solution approximately 6M in HCI.

Tris Buffer. 394 g of Tris-HCl (tris (hydroxymethyl) aminomethane hydrochloride) and 2.5 g of reagent grade NaOH are dissolved in deionized water to make 1.0 liter. This solution is 2.5 M in tris and 2.475 M in HCI, giving a pH of about 6.2 when diluted 50-fold with deionized water.

Sodium Borohydride Solution. Four grams of >98% $NaBH_4$ (previously analyzed and found to be low in arsenic) are dissolved in 100 ml of 0.02 M NaOH solution. This solution is stable 8-10 hours when kept covered at room temperature. It is prepared daily.

Phosphoric Acid Leaching Solution. To prepare 1.0 liter of 0.10 M phosphoric acid solution, 6.8 ml of reagent grade 85% H_3PO_4 are dissolved in deionized water.

Trisodium Phosphate Leaching Solution. To prepare 1.0 liter of 0.10 M trisodium phosphate solution, 6.8 ml of 85% H_3PO_4 and 12 g of reagent grade NaOH are dissolved in deionized water.

Acid Digestion Mixture. With constant stirring, 200 ml of concentrated reagent grade H_2SO_4 are slowly added to 800 ml concentrated $HN0_3$.

METHODS

Total Arsenic Determination

An aqueous sample (5-30 ml) is placed into the reaction vessel and 1.0 ml of 6M HCI is added. The 4-way valve is put in place and turned to begin purging the vessel. The G.C. trap is lowered into a Dewar flask containing liquid nitrogen (LN_2) and the flask topped off with LN_2 to a constant level. A 2.0-ml aliquot of $NaBH_4$ solution is then introduced through the silicone rubber septum with a disposable 3-ml hypodermic syringe and the timer turned on. The $NaBH_4$ is slowly added over a period of about 1 minute, being careful that the H_2 liberated by the reduction of water does not overpressurize the system or foam the contents out of the reaction vessel.

After purging the vessel for 8 minutes, the stopcock is turned to pass helium directly to the G.C. trap. In rapid order, the LN_2 flask is removed, the trap heating coil is turned on, and the chart recorder is turned on. The arsines are eluted in the order: AsH_3, CH_3AsH_2, $(CH_3)_2AsH$ according to their increasing boiling points given in Table 2.2 (1).

Table 2-2

REDUCTION PRODUCTS AND THEIR BOILING POINTS OF VARIOUS AQUEOUS ARSENIC SPECIES

Aqueous form	Reduction Product	B.P., °C
As(III), arsenous acid, $HAsO_2$	AsH_3	-55
As(V), arsenic acid, H_3As0_4	AsH_3	-55
MMA, $CH_3AsO(OH)_2$	CH_3AsH_2	2
DMA, $(CH_3)_2AsO(OH)$	$(CH_3)_2AsH$	35.6

Arsenic (Ill) Determination

The same procedure as above is used to determine arsenite, except that the initial pH is buffered at about 5 to 7 rather than <1, so as to isolate the arsenous acid by its pKa (1). This is accomplished by the addition of 1.0 ml of Tris buffer to a 5- to 30-ml aliquot of unacidified sample. (If the sample is acidic or basic, it must be neutralized first, or the buffer will be exhausted.) For the AS(III) procedure, 1.0 ml of $NaBH_4$ is added in a single short (~10 seconds) injection, as the rapid evolution of H_2 does not occur at this pH.

Small, irreproducible quantities of organic arsines may be released at this pH and should be ignored. The separation of arsenite, however, is quite reproducible and essentially 100% complete. As(V) is calculated by subtracting the AS(III) determined in this step from the total inorganic arsenic determined on an aliquot of the same sample previously.

SEDIMENTS

Total Inorganic Arsenic

A 1.00-g aliquot of freeze-dried and homogenized sediment is placed into a 100-ml snap-cap volumetric flask. Five milliliters of deionized water is added to form a slurry and then 7 ml of the acid digestion mixture is added. After 5 minutes, the caps are replaced and the flasks heated at 80 to 90°C for 2 hours. Upon cooling the samples are diluted to the mark with deionized water, shaken, and allowed to settle overnight. An appropriate-sized aliquot of the supernatant liquid (25-100 µl) is added to 20 ml of deionized water and run as for total arsenic.

Leachable Arsenite

An aliquot (~I-2 g) of fresh or freshly thawed wet homogeneous sediment is weighed to the nearest 10 mg directly into a 40-ml acid-cleaned Oak Ridge type centrifuge tube. To this is added 25 ml of 0.10 M H_3PO_4 solution and the tubes are agitated with the lids on. Periodic agitation is maintained for 18 to 24 hours, at which time the tubes are centrifuged for 30 minutes at 2500 RPM. Twenty milliliter aliquots of the supernatant liquid are removed by pipetting into cleaned polyethylene vials and saved in the refrigerator until analysis. Analysis should be accomplished within the next couple days.

For analysis, an appropriate-sized aliquot (10-100 µl) is added to 20 ml of well-characterized filtered river water (or other nonoxidizing/nonreducing water). Enough 1.0 M NaOH solution is added to approximately neutralize the H_3PO_4 (1/3 the volume of the sample aliquot), and then 1.0 ml of Tris buffer is added. The sample is then analyzed as for As(III).

Leachable Arsenate, MMA and DMA

An aliquot (~1-2 g) of wet sediment is weighed into a centrifuge tube, as above. To this are added 25 ml of 0-1 N Na_3P0_4 solution, and the tubes agitated periodically for 18 to 24 hours. After centrifugation the supernatant liquid (dark brown due to released humic materials) is analyzed as for total arsenic using an appropriate-sized aliquot in 20 ml of deionized water. The total inorganic arsenic in this case should be only As(V), as AS(III) is observed to not be released at this pH. No pre-neutralization of the sample is necessary as the HCl added is well in excess of the sample alkalinity.

Interstitial Water Analysis

Interstitial water samples may be treated just as ordinary water, except that as they are quite high in arsenic, usually an aliquot of 100 to 1000 µl diluted in deionized water or river water is appropriate in most cases.

Storage Experiments

Storage experiments designed to preserve the original arsenic speciation of samples were carried out for a wide variety of conditions. For water samples, 30-ml and 60-ml polyethylene bottles precleaned in 1 M HCI were used.

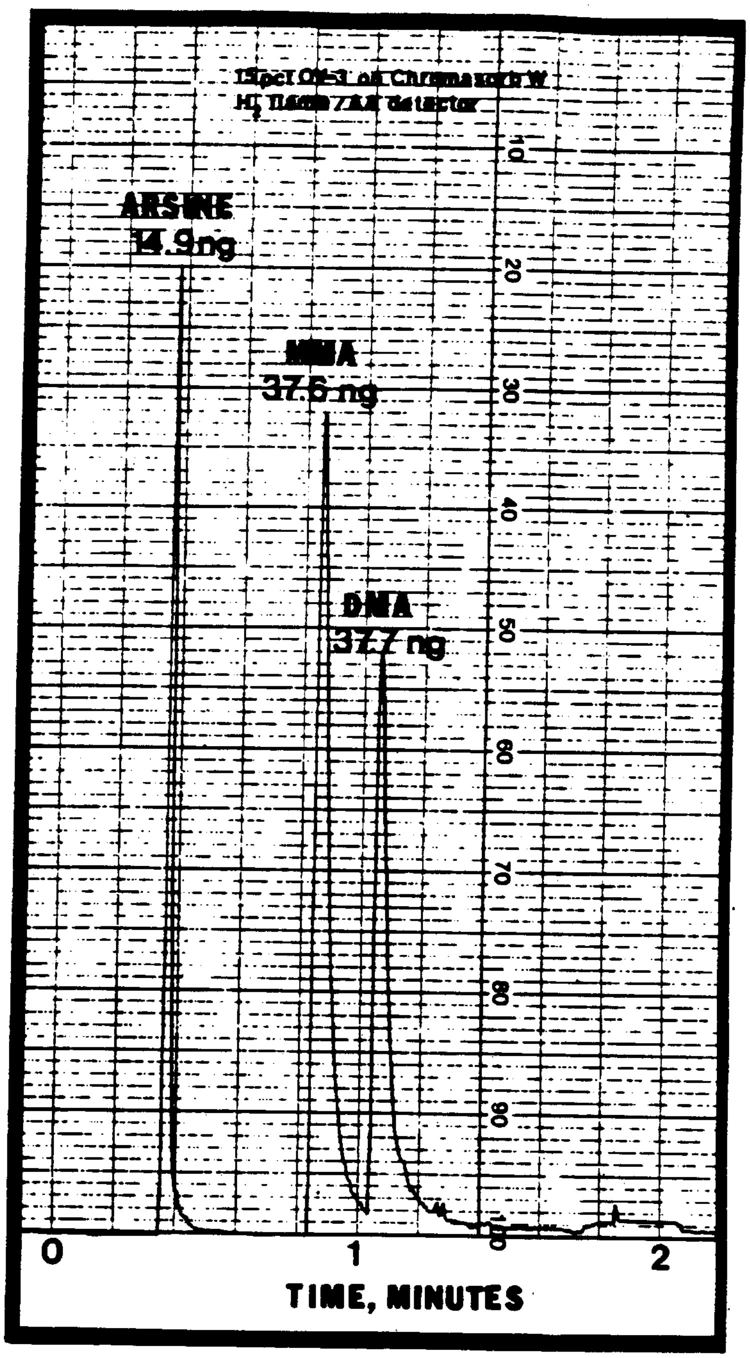
Conditions of temperature ranging from 20ºC to -196ºC were assessed, as well as preservation with HCl and ascorbic acid. Storage tests were carried out over a period of one month for water samples.

The stability of the As(III)/As(V) ratio in interstitial water at room temperature, in the presence of air was carried out over a 24-hour period to determine the feasibility of the field collection of interstitial water. Because of the time-consuming nature of sediment analysis, a two-point storage test was carried out with triplicate samples analyzed for two sediments at two temperatures (OºC and -18ºC). Mud samples were stored in polyethylene vials and analyzed at time zero and one month.

RESULTS AND DISCUSSION

Data Output

Using the procedures outlined above, and a mixed standard containing As(V), MMA, and DMA, standard curves were prepared for each of the arsines generated. A typical chromatogram from this procedure is illustrated in Figure 2.2. Under the conditions described in this paper, the elution times for the various arsines are as follows: AsH_3, 24 ± 2 s; CH_3AsH_2, 53 ± 2 s and $(CH_3)_2AsH$, 66 +/- 2 s. Notice that the peaks are broadened and that the sensitivity decreases as the boiling point of the compound increases. The small amount of signal after the DMA peak is probably a higher boiling impurity in the DMA, or some DNA that is lagging in the system during elution. We had previously noted much larger, multiple peaks in this region when water was allowed to condense between the trap and the detector. Such peaks were effectively eliminated and the DMA peak sharpened with the addition of the heating coil between the trap and the detector.



Note: Figure 2.2. Typical chromatogram of arsenic hydride species. Vertical axis absorbance, horizontal axis time.

The typical standard curves in Figure 2.3 are prepared from the mean of two determinations at each concentration. Arsenic peak-height response appears to be linear to at least 600 mau (milliabsorbance units), which is the full scale setting used on our chart recorder. Andreae (3) shows that arsenic response is extremely non-linear above this for the peak height mode, and recommends the use of peak area integration to increase the linear range. We have chosen to simply use a small enough sample aliquot to remain within 600 mau.

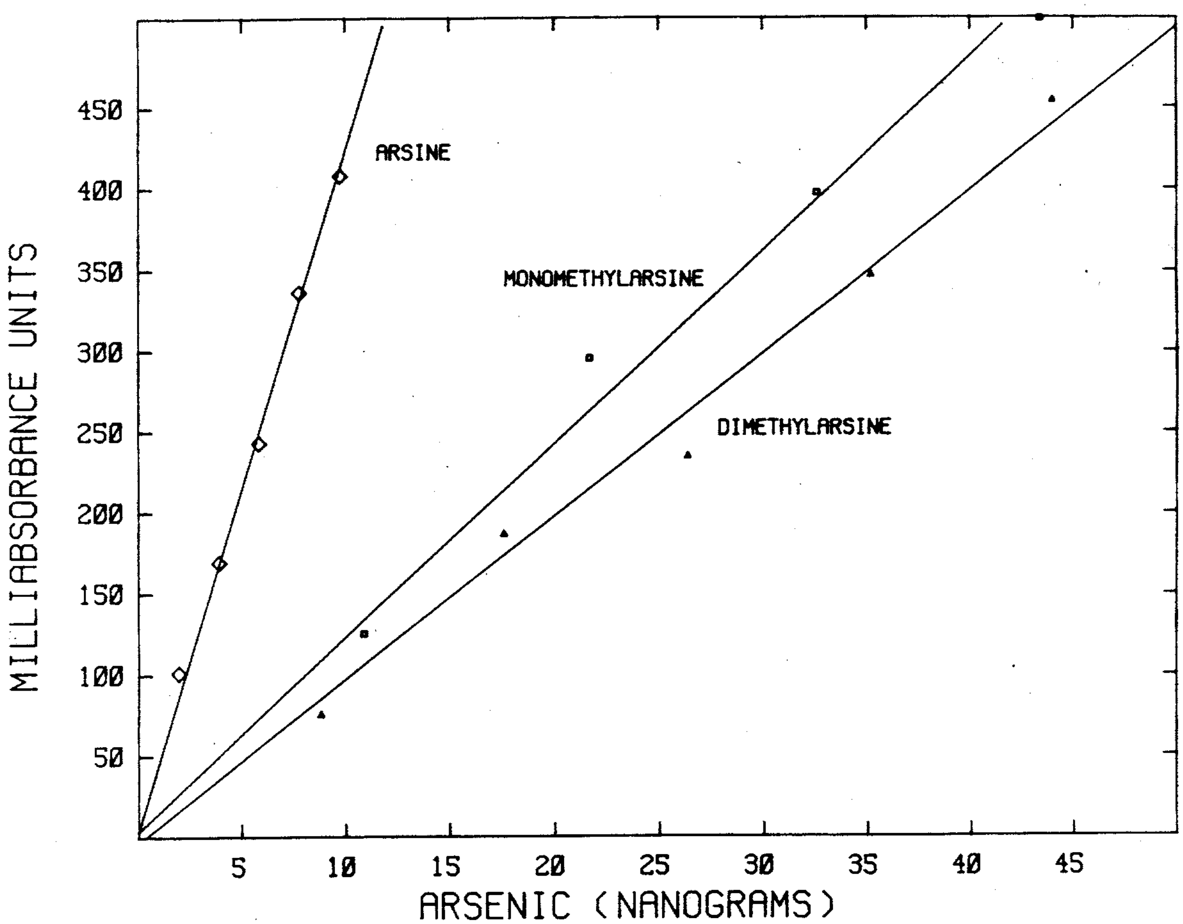


Figure 2.3. Standard curves, absorbance versus concentration for arsenic hydride species, atomic absorption detector.

As arsenic response is quite sensitive to the $H_2/0_2$ ratio in the flame, it is necessary to restandardize the instrument whenever it is set up. Usually, however, the response is quite constant and stable over the entire day.

Precision, Accuracy and Detection Limits

Precision and accuracy are the greatest and the detection limits the lowest for inorganic arsenic. The precision and accuracy of the inorganic arsenic determination is illustrated at two concentrations in Table 2-3. The standard seawater, NASS-1 (National Research Council of Canada) was run in 5.0-ml aliquots and the "standard river water" (National Bureau of Standards) was run in 100-µl aliquots. In either case, both the precision (RSD) and accuracy were about 5%. Precision begins to decrease, as the boiling point of the compound increases, as is illustrated in Table 2-4, for spiked river water. No standard reference material has been found for the organic species.

Table 2-3

REPLICATE DETERMINATIONS OF TOTAL INORGANIC ARSENIC IN SOME STANDARD WATERS

Total (inorganic) arsenic, µg•1-[1]		
Replicate	NASS-1 Seawater	NBS River water
1	1.579	81.5
2	1.556	74.5
3	1.591	71.8
4	1.493	79.0
5	1.529	79.3
N	5	5
X	1.550	77.2
S	0.040	4.0
RSD	2.6%	5.2%
Certified	1.65	76.0
±	0.19	7.0

N - number of replicates
X - mean
S - +/- one standard deviation
RSD - relative standard deviation

Table 2-4

PRECISION DATA FOR THREE ARSENIC SPECIES, ILLUSTRATING THE DECREASE IN PRECISION WITH INCREASING BOILING POINT OF SPECIES. THESE SAMPLES WERE SPIKED RIVER WATER USED IN WATER STORAGE TESTS

Arsenic concentrations, ng•1-[1]			
Replicate	Inorganic arsenic	MMA	DMA
N (8-24-83)	3	3	3
X	937	2483	2173
S	44	79	181
RSD	4.7%	3.2%	8.3%
N (9-11-83)	3	4	4
X	800	2342	2393
S	24	165	260
RSD	3.0%	7.0%	10.9%

The detection limit of this technique has not been explored to the extreme as the usual environmental sample benefits from less, not more sensitivity. For a chart recorder expansion of 600 mau full scale, and the parameters given in the text, and for a 30-ml sample aliquot, the following approximate detection limits are found: As(V), 0.006 µg•I-^{1} (twice the standard deviation of the blank); AS(III)0.003 µg•I-^{1}(0.5 chart units); MMA, 0.010 µg•-^{1} as As (0-5 chart units); DMA, 0.012µg•I-^{1} as As (0.5 chart units). For As(III), MMA and DMA, no contribution to the blank has been found due to reagents, except for the AS(III) present in the river water used as a dilutant. As for As(V) a small contribution is found, mostly from the $NABH_4$, and to a smaller extent from H_3PO_4. These may be minimized by selecting reagent lots of reagents found to be low in arsenic.

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